August 1950 Volume 4 Part 2

MENDEL DARWIN MORGAN

VAVILOV LINNAEUS DE VRIES SPALLANZANI

JOHANNSEN GALTON WEISMANN VILMORIN

HEREDITY

An International Journal of Genetics

UNIVERSITY OF MICHIGAN
SEP 21 1950
MUSEUMS LIBRARY

Edited by

Cyril D Darlington DSc FRS
Hertford

Ronald A Fisher ScD FRS
Cambridge

In collaboration with

G W Beadle
Pasadena

T Caspersson
Stockholm

Th Dobzhansky
New York

B Ephrussi
Paris

Ø Winge
Copenhagen

Oliver and Boyd
London and Edinburgh

BOVERI BATESON WILSON

INFORMATION FOR CONTRIBUTORS

Heredity publishes three times a year original articles of interest to research workers, teachers, and students in the fields of experimental breeding, cytology, statistical and biochemical genetics and evolutionary theory. New research, history, review and criticism are accepted.

MANUSCRIPTS should be prepared so as to conform with the usage in current numbers of the Journal, especially with regard to Illustrations, References, Tables, and Summaries. The address of the *Author* and the Date of Receipt of the Manuscript will be printed under the *Title* which should be scrupulously abbreviated. The following abbreviations for References will be used :—

C.R.	*Comptes rendus*
J. Genet. . . .	*Journal of Genetics*
P. Int. Gen. C. (7) . .	*Proceedings of the 7th International Genetics Congress*
P.N.A.S. . . .	*Proceedings of the National Academy of Sciences, Washington*
P.R.S., B . . .	*Proceedings of the Royal Society of London*, Series B
P.R.S. Edin., B . .	*Proceedings of the Royal Society of Edinburgh*, Series B
Z.I.A.V. . . .	*Zeitschrift für induktive Abstammungs- und Vererbungslehre*

TEXT FIGURES must be (1) suitable for line-block reproduction, (2) lettered in pencil, (3) supplied with legends and (4) separated from the text but allocated to sections of the text.

PLATES should be mounted to fit or exceed the whole size of the page ($10'' \times 6\frac{1}{4}''$) if possible. Half-tone plates can be bled if wished. Colour plates will be accepted only if the author bears half the cost of reproduction.

PROOF is supplied once, in Page form, together with an Order Form for Off-prints. Excessive corrections will be chargeable to the Author.

OFF-PRINTS are free to the number of 50. Additional copies will be supplied at cost if ordered before the issue is printed.

EDITORS :—

Dr C. D. DARLINGTON
John Innes Institution
Bayfordbury, Hertford, Herts.

Prof. R. A. FISHER
Whittingehame Lodge
Cambridge

Single Numbers, 21/- or $3.50 net
Annual Subscription, 60/- or $10.50 net

Printed and Published in Great Britain by OLIVER AND BOYD LTD.,
Edinburgh: Tweeddale Court. London: 98 Great Russell Street, W.C.

BOOKS ON THE BIOLOGICAL SCIENCES

Supplied from stock or obtained to order. Select stock of books on Biology, Genetics, Zoology, Botany, Cytology and the Social Sciences, always available. Catalogue of Works on the Biological Sciences available on request. *Please state interests when writing*

LENDING LIBRARY Medical, Scientific and Technical

Annual Subscription from ONE GUINEA
Prospectus post free on application

Bi-monthly List of New Books and New Editions sent regularly post free on request.

SECOND-HAND DEPARTMENT: 140 GOWER ST., LONDON, W.C.1.
A constantly changing large stock of Medical and Scientific books on view, classified under subjects.

Libraries or small collections purchased.

STATIONERY DEPARTMENT: Medical and Scientific Stationery.

LONDON: H. K. LEWIS & Co. Ltd.
136 GOWER STREET, LONDON, W.C.1
Telephone: EUSTON 4282 Established 1844
Business hours:—9 a.m. to 5 p.m., Saturdays to 1 p.m.

THE BASIS OF CHEMOTHERAPY

T. S. WORK, B.Sc., Ph.D.
ELIZABETH WORK, B.A., Ph.D.

Designed to give young research workers a broader and sounder basis for the study of the hybrid subject of chemotherapy, the boundaries of which stretch from organic chemistry through biochemistry and physical chemistry to bacteriology, pharmacology and therapeutics.

"The book affords the worker in closely related fields the opportunity of obtaining, in a summarised and palatable form, the results of research on the biochemistry of the living cell, and I have every pleasure in recommending it highly as accurate, authoritative, and well written."—*Pharmacy and Pharmacology.*

Medium 8vo. 556 pages. **26s.** net.

OLIVER AND BOYD LTD.
TWEEDDALE COURT - - EDINBURGH

THE WORLD'S GREATEST BOOKSHOP

FOYLES

* FOR BOOKS *

FAMED FOR ITS EXCELLENT
MEDICAL BOOKS DEPARTMENT

New and secondhand Books on every subject
Stock of three million volumes

WE BUY BOOKS, TOO

119 - 125 Charing Cross Rd., London, W.C.2.

Gerrard 5660 (16 lines) ★ *Open 9—6 (including Saturday)*

FARMING

THE JOURNAL OF
AGRICULTURAL PROGRESS

A technical journal discussing the latest scientific and technological advances in agriculture, horticulture and animal husbandry with particular reference to their practical application.

Monthly 1/6 Yearly 19/-

Specimen copies from the circulation manager, A. Hobson

JARROLD & SONS LTD.
COWGATE, NORWICH

DISCOVERY

THE MAGAZINE OF
SCIENTIFIC PROGRESS

This is Europe's leading popular science magazine. Scientists writing in non-specialist language describe their work in various branches of science and technology.

Monthly 1/6 Yearly 19/-

Specimen copies from the circulation manager, A. Hobson

JARROLD & SONS LTD.
COWGATE, NORWICH

HEREDITY VOLUME 4 PART 2 AUGUST 1950

PIGMENT SPREAD IN MAMMALIAN SKIN: SERIAL PROPAGATION AND IMMUNITY REACTIONS

R. E. BILLINGHAM and P. B. MEDAWAR
Department of Zoology, Birmingham University

Received 16.ix.49

I. INTRODUCTION

WHEN a spotted black and white guinea-pig is born, the areas of black skin bearing black hairs are incisively distinct from the areas of white skin bearing white hairs; but as it grows up, the superficial epidermis at the boundaries of the white areas is slowly, evenly, and progressively encroached upon by pigment. The white hairs are unaffected. In an adult spotted guinea-pig, therefore, the areas of black skin bearing black hairs are separated from the areas of white skin bearing white hairs by a transitional band of black skin bearing white hairs—a band which may reach about one cm. in breadth by a combination of ordinary growth and "pigment spread."

Pigment spread occurs in several other animals: in the body skin of Friesian cattle (see below) and spotted pigs, and in the tails of recessively spotted mice (Reynolds, unpub.); moreover, as we shall show below, red, chocolate, and doubtless other pigments will likewise encroach upon white skin, though one pigmented area in a parti-coloured animal will only encroach upon another at an altogether lower order of rate. In all such cases, pigment spread is accelerated and made accessible to experimental analysis by grafting operations: for example, by the grafting of black skin into white or white into black.

In our first analysis (Billingham and Medawar, 1948*a*), we considered four hypotheses that might account for the phenomenon of pigment spread, and dismissed two of them—that spread is the consequence of an invasive replacement of a white by a pigmented epithelium, or of a mere leakage or diffusion from black skin into white of a factor required for the initiation or maintenance of pigmentary activity. Two further possibilities remained on probation.

According to the hypothesis of *melanophore migration*, pigment spread is the outcome of a differential migration from black skin into white of the melanophores (pigmentary dendritic cells) * that are the acknowledged origin and seat of the pigmentary activity of

* The term "dendritic cell" is wider in scope than "melanophore" since it may refer to a non-pigmentary cell.

skin. (By " differential migration " is meant the migration of melanophores independently of the epidermal cells to which they normally supply pigment.) This explanation, though not disproved, required the acceptance of a variety of auxiliary hypotheses to sustain it.

The authors' observations and experimental results were, however, directly and adequately accounted for by a fourth hypothesis : that pigment spread involves an *infective cellular transformation.* Gold impregnation methods (Billingham, 1948) had revealed in white skin a system of dendritic cells similar to the melanophores of pigmented skin in anatomy, density, and disposition : these " white " dendritic cells contain no melanin and lack the enzymic equipment required for making it. The entire system of epidermal dendritic cells—the " epidermal glial system " of the skin—forms a partly anastomising and therefore in some degree syncytial reticulum. It is moreover a perhaps unique property of the pigmentary dendritic cell that in some manner it " inoculates " formed pigment granules into the Malpighian cells to which its branches are intimately applied—a type of activity that Masson (1948) has called *cytocrine.*

The fourth hypothesis under discussion accordingly asserts that pigment spread depends upon the inoculation or infection of white dendritic cells by a cytoplasmic ingredient derived from their pigmentary neighbours at the boundary zone between black and white skin where dendritic cells of these two types may come into anatomical contact. The transformation of a white into a pigmentary dendritic cell is permanent in cellular heredity, and a white dendritic cell so transformed may in turn transform its white neighbours. Pigmentation therefore spreads in an evenly advancing front in a manner formally equivalent to a virus infection.

The rate and pattern of pigment spread depend upon the number and distribution of the dendritic cells in the skin that is undergoing infection. The fact that tongue epithelium when transplanted to black body skin remained permanently unblackened was adequately accounted for by the fact that gold impregnation methods had wholly failed to reveal a system of dendritic cells within it ; and we have since shown that a black skin graft transplanted to the tongue, while retaining its full specificity of histological type, fails completely to initiate pigment spread for the same reason (plate I, figs. 1 and 2). Moreover, the epidermal glial system, though continuous in the basal layer of the epidermis, is sharply interrupted at the necks of the hair follicles ; and it is common knowledge that the hair bulbs and hairs may have a colour system phenotypically different from that of the surface of the skin. The fact that white hairs are normally uninfluenced by pigment spread in the epidermis above them is thus adequately explained by absence from the follicle neck of the dendritic cells required to mediate its passage. These facts, and certain others of smaller weight, are more directly explained by the hypothesis of an infective cellular transformation than by that of melanophore migration.

Section 2 of the present paper deals briefly with the inter-relationships between the pigmentary systems of black, red and white skins. Section 3 contains an experimental analysis of the propagation of pigmentary " infection " from one guinea-pig to another. Although nothing in either section can be regarded as an outright refutation of the melanophore migration hypothesis, both describe phenomena that are all but impossible to reconcile with it. Since the majority of our experiments were done with the object of discriminating between the migration and infection hypotheses, and may seem pointless without this background in mind, the " Discussion " of results that is usually relegated to the end of a paper has been incorporated in the body of the text.

2. PIGMENTARY RELATIONSHIPS BETWEEN BLACK, RED, AND WHITE SKINS

Studies by the " skin-splitting " method of Billingham and Medawar (1948*a*) have shown that black, red and chocolate skins differ from each other neither in the number nor the mode of distribution of their melanophores, but only in the pigmentary activity of the individual cells. (The same is true of the difference between negro and white human skin : Billingham, 1949.) Melanophores of each such type are " true breeding " and each is a member of a distinct somatic cell lineage.

Red and chocolate pigments both spread into white skin, whether from grafts or across natural colour boundaries. No particular study has been made of chocolate-coloured skin, but with each of five red-coloured autografts transplanted to white skin areas and watched for from 350 to 550 days, it has been found that pigment spread takes longer to start than with a black-in-white graft and thereafter proceeds at only about two-thirds of its rate (plate I, figs. 3 and 4). Experiments have now been begun which will make it possible to compute the difference more accurately and to correlate the rate of spread from red-in-white grafts with their depth of pigmentation. It is clear that if pigment spread is due to melanophore migration, then red melanophores must be thought to be in some way physiologically feebler than black. If it is due to a spreading infection process, then one possibility is that the infective pigmentary enzyme complex of the cytoplasm is less abundant in red skins. The positive correlation between pigment volume and colour intensity in mouse skins (Russell, 1948) lends somewhat indirect support to this view.

Three methods have been used to investigate the capacity of black pigment to spread into red-coloured skins elsewhere on the same tri-coloured guinea-pig.

(a) *Black to red transplantation*

Of five animals bearing black-in-red grafts (plate II, figs. 6, 7 and 8), one died on the 421st day after operation and the remaining

four are still under observation at the 620th day. Only one (plate II, fig. 8), that in which the red skin surrounding the black graft was conspicuously the palest, gave evidence of pigment spread. There had been some inconclusive evidence of the blurring of what should normally be an incisive graft outline after 56 days ; and a 1 mm.-wide annulus of spread recorded at 295 days had increased to a width of about 2½ mm. by 620 days. In this one animal, therefore, pigment spread has been occurring at the mean rate of about 1½ mm. per annum—a rate hitherto without precedent.

(b) Red to black transplantation

In one guinea-pig, a very pale red-coloured ear-skin graft was transplanted from the dorsum of the left ear to an area of black body skin on the chest. Inward spread of pigment was just perceptible by the 65th day after operation and conspicuous by the 233rd : it went on at a mean rate of about 2 mm. per annum until by the 555th day after operation only an irregular patch of pale red skin about 1 mm. in width in the centre of the graft remained to be encroached upon (plate II, fig. 5). The rest of the graft was identical in colour with an originally black graft cut from the right ear of the same animal on the same occasion and transplanted to an area of white skin.

The epidermis from four samples of skin was at this stage removed and examined microscopically in the living state and after a short treatment with 1 : 1000 dihydroxyphenylalanine (" Dopa ") in phosphate buffer at *p*H 7·4. These were : the original red-in-black graft, now all but wholly black ; the original black-in-white graft ; a sample from the dorsum of the pale red-coloured left ear from which the red graft had been cut 555 days beforehand ; and a sample from the dorsum of the black right ear, from which the black graft had been cut 555 days beforehand. (The purpose of the " Dopa " treatment was only to render the very faint melanophores of the red skin sharply distinct.)

The epidermal patterns and the number and distribution of the melanophores were the same in all four specimens. But the originally weak red-coloured melanophores from that part of the red-in-black graft which had been encroached upon by black pigment were now fully black (plate II, figs. 9, 10 and 11), and each one was surrounded by Malpighian cells heavily inoculated with pigment granules. In this area, *no* red melanophores were present ; and the melanophores that were present were in no way distinguishable, whether in number or pigmentary activity, from the black melanophores of the black-in-white graft or of the right ear. The small central patch of the red-in-black graft that had still to be encroached upon by the advancing front of black pigment contained weak red-coloured melanophores *only* (plate II, figs. 12 and 13), and these were indistinguishable in any way from the melanophores of the skin of the left ear. Nowhere in the specimen were black and red melanophores found to be mixed.

These results seem to admit of only one interpretation : that the originally red-coloured melanophores of the red-in-black graft had been serially transformed into black melanophores from the periphery of the graft inwards. No single observation we have made seems so damaging to the hypothesis that pigment invasion was on this occasion the consequence of a migration of black melanophores into red skin.

A second guinea-pig, operated upon in exactly the same way, was lost 214 days after operation, but not before giving clear evidence of the beginnings of the process which in the preceding guinea-pig had passed almost to completion. In this guinea-pig, too, the red-in-black graft was originally very pale in colour.

(c) *Black and red to white transplantation*

Five guinea-pigs in which red and black grafts had been transplanted close enough together on white skin to allow the areas of pigmentation spreading from them to coalesce (plate I, figs. 3 and 4 ; plate II, fig. 5) have not so far, after 552 days, given any evidence of interaction between pigments of the two types. On the contrary, the pattern of spread is such that the more rapidly advancing black pigment gives the appearance of avoiding the zones earlier tenanted by red pigment spreading from the red grafts (plate II, fig. 5).

The experimental results outlined above show that black pigmentation may indeed spread into red skins ; and they suggest, at present only very roughly, that the rate of spread of a dark pigment into a white or lightly pigmented skin varies directly with the difference between their degrees of colouration. A discussion of the relevance of these facts to the infection hypothesis will be deferred until more exact quantitative information has become available.

3. THE PROPAGATION OF PIGMENTARY "INFECTION" FROM ANIMAL TO ANIMAL

In our earlier paper on the phenomenon of pigment spread, we reported seven trials in each of which even a very small graft of black skin taken from one guinea-pig failed to initiate pigment spread after transplantation immediately below the white skin of another ; and although attention was drawn to certain technical shortcomings of these trials, we felt that our failure could more properly be attributed to the immunological disparity between genetically heterogeneous guinea-pigs than to inadequacy of technique. In this section it will be shown that our failure to transmit pigmentation from one guinea-pig to another was wholly technical in origin, though the *persistence* of foreign pigmentation will at the same time be shown to be entirely dependent upon the immunological relationship between donor and recipient.

The guinea-pigs used in the experiments described below came from dealers' miscellaneous stock ; supplies were replenished from

time to time by new purchases or by the offspring of pen breeding. From the genetical standpoint they can be regarded as a very heterogeneous assembly.

(i) *Operative methods : the seeding graft*

The cells of skin and of many other tissues do not survive transplantation between individuals of ordinary heterogeneous stocks : cells so transplanted provoke an unusual type of immunity reaction and survive for a length of time that varies inversely with the degree of genetic diversity between donor and recipient and the quantity of foreign tissue that is grafted.* Attempts to transmit the factor responsible for pigment spread by dead cells or cell-free extracts have, however, so far failed (see section 4), and pigmentation that is to be propagated from one guinea-pig to another must therefore be initiated by living foreign cells. (Whether after its initiation pigment spread is *maintained* by surviving foreign cells is a crucial problem to be discussed in full below.)

If melanophores from one guinea-pig are to initiate pigment spread in the white skin of another, two technical conditions must be satisfied : (*a*) the " dosage " of foreign cells transplanted from donor to recipient must be exceedingly small, and (*b*) the foreign pigmentary dendritic cells must be caused to enter very rapidly into intimate contact with their white analogues in the recipient's skin. The success of experiments which satisfy these two conditions is, in our interpretation, to be attributed to the fact that, being in very low dosage, the foreign cells have time to " infect " the host cells before their eventual destruction.

In practice, the " grafts " we have used are suspensions of basal-layer epidermal cells in Ringer's solution, handled and applied by pipette. A pigmented ear is by far the most easily workable source of skin for this purpose. A thin vaselined shaving, about 0·5 cm.^{2} in area, is incubated in sterile trypsin solution (see Billingham and Medawar, 1948*a*) until the dermis may be cleanly peeled away, so leaving the epidermis behind as a single intact sheet. The epidermal sheet is smoothed down on its vaselined cuticular surface and its basal-layer then scraped off by smooth strokes with the blade of a cataract knife. Keratinised cells and the cells of all the more superficial epidermal strata are left behind. The basal-layer scrapings are coarsely broken up and suspended in Ringer's solution by sucking them in and out of a fine pipette. In this condition the preparation awaits use.

The recipient area or " graft bed " to which such an emulsion may be transferred is essentially that area which is left behind after the removal of a Thiersch graft ; *i.e.* the surface of the skin is removed by one or two clean scalpel strokes to such a depth as will expose

* For the behaviour of skin homografts, see Medawar (1944, 1945, 1946*a*, *b*, 1948*a*, *b*). Evidence that many other tissues do not survive homoplastic transplantation is to be found in Loeb (1945).

the bases of the hair follicles (fig. 1). Areas of this sort (unlike full-thickness defects of skin) heal very rapidly, without contracture, and with cosmetically admirable results by the multiple upward emigration of epidermal epithelium accompanied by dendritic cells from the hair follicles. In doing so, the native cells mix rapidly and intimately with such foreign cells as may have been transferred to the surface of the raw area by pipette. Moreover, the rapid epithelial closure of the graft bed to a large extent prevents the seeded cells from increasing their initial dosage by proliferation.

We have come to describe such an operation as a *seeding graft*. If the cells seeded are of native (*i.e.* autologous) origin, there are only technical limits to the area of the recipient bed and the number of cells that may be seeded on to it. When the seeds are of foreign origin, the area of the bed should not exceed 0·5 cm.^{2}, and the quantity of epidermal matter transferred to it should not exceed that equivalent to 2-3mm.^{2} of skin. In theory, this may be too high a dosage, but in practice a sufficiently high proportion of the seeded

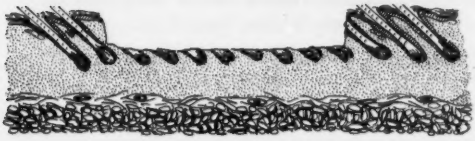


FIG. 1.—Sectional diagram illustrating the depth to which the skin must be cut in the preparation of a seeding bed.

FIG. 2.—Sectional diagram illustrating the area and depth of the smaller type of seeding bed.

cells die. The preferred site for a seeding bed, as for orthodox grafts, is the skin of the side of the chest. After the excess of Ringer's solution has been carefully drained or pipetted away to leave the epidermal seeds thinly and evenly scattered over its surface, the seeding bed is covered by two or three thicknesses of extra fine-mesh vaselined muslin. The thorax is thereupon directly wound with two or three turns of plaster bandage, which is left on until the first post-operative inspection 7-10 days later.

Another type of bed is preferred when for any reason the quantity of cells to be grafted is very small : this is in effect the raw area left behind when a very small " pinch " graft (as opposed to a Thiersch graft) is cut from the skin of the recipient. Fig. 2 makes its structure clear.

It is sometimes obligatory to suspend the basal-layer cells of pigmented *body* skin for propagation, as when an area of pigment spread induced in a second animal by cells from a first is to be transmitted to a third, and then in turn to a fourth, and so on—" serial propagation." The epidermis of body skin is thinner than that of ear skin, and the elasticity of the dermis makes it much more

difficult to cut grafts of even thickness. With increasing experience we have come to succeed with it regularly, provided that the body-skin shaving is fully stretched and then gummed on to a coverslip with rubber-paraffin grease before immersion in trypsin solution for the skin splitting operation. Body skin is more sensitive than ear skin to over-digestion with trypsin, which causes the basal-layer cells to macerate. An accurate timing of the digestion process is therefore particularly necessary.

Many spotted guinea-pigs do not have white skin conveniently placed on the chest region (*cf.* plate IV, figs. 25 and 26) for the seeding operation. By using rubber puncture-patches or cellophane dressings sealed down with "Portex" plastic skin, seeding operations may, however, be done on the sacrum, thigh or abdomen. On four occasions, with successful results on three, seeding homografts have been transplanted to the sole of the foot (plate IV, fig. 31) by using a plaster "sock" as a temporary dressing. But for reasons that will be clear later the abundance of its hair follicles makes body skin superior to any other as an epidermal seed bed.

The latest analysis of our experimental results shows that seeding operations have failed for technical reasons in less than 8 per cent. of trials, but there are a good many reasons for supposing that some of the negative results attributed to technical failures were in reality due to immunological reactions (see below).

(ii) *Inspections and dressings*

Plasters and other dressings may be removed between the 7th and 10th days after operation, by which time the originally raw area of the seed bed has been fully resurfaced by skin epithelium from the hair bases. A second dressing to last until the 15th or 20th day is desirable only inasmuch as it prevents the guinea-pig from scratching the skin of the operation field.

A successful primary "take" is revealed by the presence in the healed graft bed of a variable number of small, rounded, and pale leaden-blue spots with indistinct outlines, but a seeding operation has in some cases proved to be successful even when such spots were not visible at the first inspection, since the rapid cell division associated with the later stages of healing entails a high degree of pigment dilution. Between the 10th and 20th day the spots deepen in colour and coalesce more or less completely to form a tracery of pigmentation over the surface of the original graft bed; and with very rare exceptions a variable number of stout, fully pigmented hairs will be found to have pierced the skin surface, since this method of transplantation gives the seeded melanophores the direct access to hair follicles which is denied them by ordinary methods of grafting.

Since seedings with foreign cells may fail for immunological as well as technical reasons, we have, however, thought it right to use

more exacting criteria of primary " take " than those described above. A homograft seeding is admitted to be technically successful only if at least one of the following three criteria is satisfied : (*a*) the darkening between first and second inspections of the spots that represent the primary foci of pigmentation ; (*b*) the formation of at least one black hair ; or (*c*) the initiation of pigment spread. The only homograft seedings admitted into the Survival Table computed in section (iii) are those which have satisfied these more exacting criteria of primary take.

Once begun, pigment spread proceeds just as if it had been initiated by a large and cosmetically perfect graft (for a full description, see our article, 1948*a*). A seeded area may be distinguished from a grafted area because the centre of origin of pigment spread is represented only by a few streaks of hyperpigmented skin bearing black hairs—the remains of the primary foci of pigmentation. The spread of pigmentation into normal undamaged skin round the seeding bed leaves the white hair follicles untouched. The peculiarities of spread initiated by seeding operations are better illustrated than described : *cf.* plate II, fig. 14 ; plate III, figs. 15, 16 and 21, etc.

We have made fully annotated inspections of animals carrying seeding grafts every 10th day up to the 50th or 70th day, and thereafter at 20 or 30 day intervals.

(iii) *The fate of seeding grafts of foreign origin*

A certain proportion of pigmented patches induced in one guinea-pig by pigmented cells from another do not long survive their first establishment on foreign soil : such patches undergo a more or less prolonged process of progressive bleaching, the first signs of which (*cf.* plate III, fig. 20) can be detected with some precision. The end result of a complete bleaching process—not all pass to completion—is the re-establishment of normal white skin in an area which had earlier been deeply pigmented. The " expectation of survival " of a homograft seeding has been computed by ordinary actuarial methods from the data which immediately follow. Needless to say, these data exclude animals in which bleaching was artificially brought about by immunological methods (section iv).

Of 79 animals which, after seeding with foreign pigmented skin cells, satisfied our accepted criteria of primary take, only 48 still bore perfectly normal patches of pigmentation by the 50th day. The remaining 31 had either lost their induced pigmentation by that time, or had shown the beginnings of a bleaching process which later inspections proved to pass to completion.

Of 48 animals which still bore patches of normal pigmentation at the 50th day, four were used for immunisation experiments which involved a deliberate bleaching out of foreign pigmentation, three were accidentally killed, and one died, leaving 40. Of these 40, 33 continued normal to the 100th day after operation, and the remaining 7 began to undergo bleaching.

Of 33 animals which still bore patches of normal pigmentation at the

100th day, six had not reached the age of 150 days after operation when this record was compiled on 1st September 1949, and two died, leaving 25. Of these 25, 23 continued normal until the 150th day after operation and the remaining two began to undergo bleaching.

Of 23 animals which still bore patches of normal pigmentation at the 150th day, two had not reached the age of 200 days after operation when this record was compiled, three were used for immunisation experiments, and one died, leaving 17. Of these 17, 16 continued normal until the 200th day after operation and one began to undergo bleaching.

Of 16 animals which still bore patches of normal pigmentation at the 200th day, two had not reached the age of 250 days after operation when this record was compiled, one was used for immunisation experiments, and one died. The remaining 12 continued normal until the 250th day.

Of 12 animals which still bore patches of normal pigmentation at the 250th day, five have not reached the age of 300 days after operation, and one has died. The remaining six have continued normal until the 300th day. (At present two animals still bear pigment of foreign origin at 350 days and one at 400 days after operation.)

From these data we may directly compute the " specific mortality " of patches of induced pigmentation of foreign origin, *i.e.* the proportion of homograft seedings found to have *begun* to fade out by 50, 100, 150, . . . 300 days expressed as a percentage of those that had been perfectly normal 50 days earlier. (Since the bleaching process may take 100 days or even more from start to finish, the tables which follow have been based, not on the total survival time of pigmentation of foreign origin, but on its survival in a state of perfect normality. This is analagous to computing actuarial statistics, not from the ages of death, but from the ages of onset of diseases which sooner or later proved fatal.)

TABLE 1

50	days	39	per cent.	based	on	79	animals	
100	,,	16	,,	,,	,,	,, 40	,,	
150	,,	8	,,	,,	,,	,, 25	,,	
200	,,	6	,,	,,	,,	,, 17	,,	
250	,,	0	,,	,,	,,	,, 12	,,	
300	,,	0	,,	,,	,,	,, 6	,,	

From these in turn may be directly computed the " expectation of survival at birth " of pigmentation of foreign origin, *i.e.* the percentage of homograft seedings which, having given clear evidence of sound primary healing, may be expected to remain perfectly normal for 0, 50, 100, . . . 300 days (see fig. 3) :—

TABLE 2

0	days	.	.	.	.	.	.	100	per cent.
50	,,	.	.	.	.	.	.	61	,, ,,
100	,,	.	.	.	.	.	.	50	,, ,,
150	,,	.	.	.	.	.	.	46	,, ,,
200	,,	.	.	.	.	.	.	43	,, ,,
250	,,	.	.	.	.	.	.	43	,, ,,
300	,,	.	.	.	.	.	.	43	,, ,,

Finally (fig. 4) we may compute the expectation of survival to (say) 300 days of homograft seedings which have survived in a state of perfect normality to 0, 50, 150, . . . 300 days :—

TABLE 3

0 days	. . . . .	43 per cent.
50 „	. . . . .	71 „ „
100 „	. . . . .	86 „ „
150 „	. . . . .	94 „ „
200 „	. . . . .	100 „ „
250 „	. . . . .	100 „ „
300 „	. . . . .	100 „ „

Two theoretically important inferences may be drawn from the preceding table : (*a*) that the expectation of survival of pigmentation of foreign origin increases with its age from inception, and (*b*) that

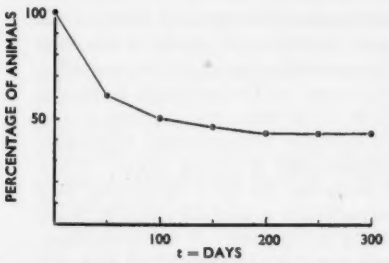


FIG. 3.—The "survival curve" of induced pigmentation of foreign origin : showing the percentage of animals in which pigmentation of foreign origin persisted in a state of perfect normality for $t = 0, 50, 100, \ldots 300$ days after its inception.

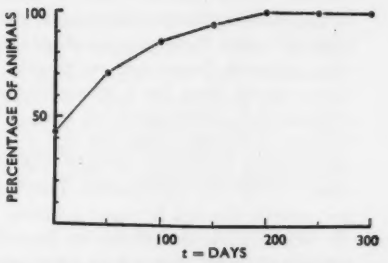


FIG. 4.—The "expectation of survival" of induced pigmentation of foreign origin : showing the percentage of animals in which foreign pigmentation of $t = 0, 50, 100, \ldots$ 300 days' standing proved to survive in a state of perfect normality for 300 days after its inception.

the expectation of survival increases in spite of the fact that during the whole period of its residence *the quantity of foreign pigmentary matter is itself steadily increasing*. A successful homograft seeding begins as two or three minute pigment foci representing the survivors of the foreign epidermal cells taken from 2 mm.^{2} of skin or less. After 250 days the area of a successful homograft seeding may well have increased by natural pigment spread and artificial expansion (see below) to an area between 250 and 350 mm.^{2} Yet the former stands a much lower chance of surviving a further 50 days than the latter. It is extremely difficult to reconcile these facts with the hypothesis that pigmentation of foreign origin is caused by the survival, proliferation and outward migration of pigmentary dendritic cells. This interpretation requires us to believe not only that foreign dendritic cells are in high degree exempt from the ordinary exactions of tissue transplantation immunity, but also that their degree of exemption rises as they increase in number. Both these subsidiary hypotheses are possible, but neither is likely.

It might be that the foreign cells progressively desensitize their hosts, as guinea-pigs may be desensitized to foreign protein, but the evidence of the next section shows that they do not.

It is obviously a matter of some difficulty to appraise the significance of a mortality table (table 1) in which each successive entry is of necessity computed only from that fraction of the original fully heterogeneous population which still bore normal pigmentation of foreign origin 50 days beforehand. It is, however, possible to work out what form the survival curve (fig. 3) would take if the foreign pigmentation were indeed due to the survival and proliferation of foreign cells. This may be done in two stages: (*a*) computing its form if the foreign cells remained in constant dosage throughout their period of residence, and then, (*b*) superimposing upon this the effect of a steadily increasing dosage of foreign cells.

(*a*) Other things being equal, the expectation of survival of foreign cells depends upon the antigenic disparity, and therefore the genetical relationship, between donor and recipient. The successive entries in the mortality table would then be a straightforward record of the selection from the original fully heterogeneous stock of those recipients most tolerant to the survival of foreign cells, and fig. 3 would illustrate a transformed tolerance-distribution curve for foreign cells in guinea-pigs of mixed stock. What form would this distribution take? It is agreed by all investigators that the genetic control of tissue grafting compatibility is multifactorial in type. (It would be more correct to describe it as polygenic.) If this is so, then the distribution through a heterogeneous stock of the lengths of time for which the recipients can just tolerate the survival of foreign cells transplanted from their donors should approximate to the normal, or should at least be bell-shaped; *i.e.* the proportion of animals which can just tolerate the survival of a uniform dosage of foreign cells for a mean length of time x days will be higher than that which can tolerate it for a shorter time $x-y$ days or a longer time $x+y$ days. If this assumption is correct, then it follows directly that the survival curve which in fig. 3 is seen to fall uninflected at a steadily diminishing rate must be sigmoid, and must fall at a steadily increasing rate down to a point of inflexion beyond which it falls at a decreasing rate. The evidence of Medawar (1944) shows that the underlying assumption is indeed correct: the survival curves of skin homografts transplanted in uniform dosage between members of a fully heterogeneous stock of rabbits are invariably sigmoid in just this way.

(*b*) Other things being equal, the survival time of foreign cells varies inversely with the dosage in which they are transplanted (Medawar, 1944, 1945). The effect of a steadily increasing dosage of foreign cells would therefore be to expedite their breakdown and so to exaggerate and prolong the tendency of the sigmoid survival curve to fall at an increasing rate. In the extreme case, if the dosage factor came to outweigh the effect of the genetic disparity between donor and recipient, the curve would be uninflected and would be such that it fell throughout at a steadily increasing rate. In other words, its properties would be exactly the opposite to those of the asymptotically declining survival curve (fig. 3) actually computed from the data.

In summary, then, the hypothesis that pigmentation of foreign origin is due to the survival and proliferation of foreign cells is very difficult to reconcile with the numerical properties of the mortality table.

A seeding of foreign origin that may be described as " perfectly normal " is one which differs in no respect, outwardly or to histological examination, from one of autologous origin (see plate III, figs. 15-19). Nevertheless the majority of homograft seedings which survive for 100 days or more go through a fairly well defined " bad period " between 30 and 50 days after their initiation. During this period, the heavily pigmented epithelium of the primary seeding foci tends to become weaker in colour or even smokily translucent, and the pigmentation in the area of new spread into normal skin becomes more dilute in colour and less crisply reticular in pattern. Histologically, this period is associated with a mild inflammation under the seeding centres, with dilatation of blood vessels and lymphatics and some infiltration of the dermis by round cells. Our belief, technically almost impossible to verify, is that this transient bleaching period corresponds with the completion of the homograft reaction provoked by the originally minute dosage of surviving foreign cells, and that the pigmentation which survives this transient bleaching phase is caused by a foreign melanogenic system within dendritic cells of native origin.

In five out of about 100 experiments in which foreign pigmentation was initiated by epidermal suspensions prepared from black ear skin, a small crop of bold red-coloured or straw-yellow hairs were found to have pierced the epidermis of the primary seeding foci between the 20th and 50th days. The lack of a comparable number of autograft seeding controls studied with this phenomenon in mind, and the possibility that the seeding material was contaminated by small numbers of red melanophores, makes it impossible to attach much significance to this observation at present.

The process of bleaching in a well-established seeding begins with a general paling or browning of the surface pigment associated with the development of curious and characteristic vacuolar defects, so that the area of pigment spread becomes coarsely patchy instead of sharply reticular (plate III, fig. 20). The rather thicker epidermis over the primary seeding foci fades out progressively, becoming at first smoky in colour and then quite translucent, so that the shafts of pigmented hairs may be seen through it. Except for a long-persistent surface smuttiness that is due solely to tattooing, *i.e.* to the retention within the dermis of histiocytes containing ingested melanin granules, the bleaching of the surface epithelium takes place within 15-30 days (*cf.* plate III, fig. 22). Pigmentation in the hairs persists very much longer—in some cases for well over 100 days, though during this period both the number and the stoutness of the hairs progressively diminishes. It is a matter of empirical fact that the melanophores

within the hair bulbs are for some reason in an immunologically privileged position.

In a small proportion of animals the immunity reaction which starts the bleaching process is just so balanced that it does not pass to completion, and a reduced number of black hairs continues to grow through a perfectly white surface epithelium. In three such cases, after periods ranging from 110 to 144 days after the beginning of the reaction that led to bleaching elsewhere than in a few hair bases, the surface epithelium was caused to be re-colonised by melanophores merely by shaving off the surface of the skin to enforce an upward migration of epithelium from the follicles. Pigment spread thereupon began anew and continues to the present time, not less than 60 days after its second inception. (This experiment, taken in conjunction with the consequences of the seeding operation itself and a variety of other evidence, shows that the dendritic cells of hair and superficial epidermis are mutually interchangeable.) In two other such cases, however, the same operation merely expedited the total loss of pigmentation, presumably because the recipient's immune state was still in being when the melanophores were caused to emerge from their follicles.

The preceding paragraphs are concerned with the course of events during the fading-out of a well-established seeding of foreign origin. Early fade-out, such as may be in progress as soon as the 20th day after operation, requires no special comment, save to point out that here too the pigmentation of the hairs long outlives that of the surface epithelium.

No form of trauma or operative interference will bring about depigmentation in an established normal homograft seeding. It has often been necessary to remove the whole or the greater part of the surface epidermis from such a seeding, either for histological examination or to propagate the induced pigmentation to another animal. Pigment spread is thereupon re-established by the upward migration of pigmented epithelium from the hair bases or from the residual surface pigmentation, or both. The operation we have called "expansion" takes advantage of this property: its object is to increase the total area of foreign pigmentation by quicker means than waiting for natural pigment spread. The operation involves no more than removing a number of Thiersch or pinch grafts from within the perimeter of an established area of pigment spread and then transplanting them in a ring or in some other convenient position in the normal white skin around it (plate III, fig. 23; plate IV, figs. 24 and 25). Each secondary graft acts as a centre of origin of pigment spread which unites with that arising from the parent seeding and thus rapidly and greatly increases the total quantity of foreign pigmentary matter. On two animals expansion operations were done twice with intervals of 50 or 60 days between each, and we have no evidence that the process cannot be repeated indefinitely. In this

manner the area of foreign pigmentation has so far been raised to 350 mm.^{2} or more.

Another method of expediting pigment spread, used with success on each of three trials, is to cause the foreign pigmentation spreading in body skin to " infect " white ear or sole-of-foot skin autografts transplanted to its neighbourhood, for pigment spread, as we found, is more rapid in ear and sole skin than in body skin (1948*a*). It is noteworthy that pigmentation induced by this means in, for example, ear skin, is very much more intense than in the body skin from which it arose in the first instance. This is another of those individually trivial but cumulatively impressive facts that argue against the idea that melanophore migration is the cause of pigment spread.

Although black pigmentation to be propagated from one spotted guinea-pig to another must for all practical purposes be induced in a white skin area on its intended recipient, it is not necessary that the pigmented areas of the recipient should themselves be black. Widespread and (to the present time) enduring pigmentation has been induced in guinea-pigs of the phenotypes red-and-white (plate IV, fig. 26) and chocolate-and-white (plate IV, fig. 24). Pigment spread that was on one occasion propagated to an animal of phenotype agouti-and-white spread abnormally slowly, but progressively nevertheless.

We have now begun a series of experiments making use of guinea-pigs of the so-called " albino " phenotype but coloured genotype.* On each of eight trials, an epidermal suspension prepared from black ear skin was seeded to recipient areas cut from the chest skin of albinos. In five, the seeding foci were perceptibly discoloured by the first inspection at the 9th day ; in none did any trace of pigmentation whatsoever survive until the 29th day. This disappearance of pigment is more rapid than can be plausibly accounted for by the destruction of the very small quantity of foreign cells in the ordinary course of the homograft reaction. Its physiological mechanism is now under investigation. Five strictly controlled experiments failed to reveal that the seeding of albino epidermal cells to black areas of chest skin has any effect on the native pigmentation.

One further experimental result provides a transition between this section and the next. An animal which has proved to be resistant to inoculation by foreign cells on one occasion, in the sense that the induced foreign pigmentation has more or less rapidly faded out, is in the same sense totally resistant to inoculation by foreign cells from the same donor source on a later occasion. Ten spotted guinea-pigs in which foreign pigmentation had faded out so rapidly that in some there was reasonable doubt about the technical success of the

* As is well known, the type of guinea-pig commonly called " albino " is a pink-eyed animal of extremely dilute pigmentation whose points darken after prolonged exposure to cold weather. Its dendritic cells are dopa-positive throughout and, like the weakly pigmentary dendritic cells of white human skin (Billingham, 1948), stain supravitally with methylene blue.

seeding operation were for a second time seeded from their original donors after periods ranging from 91 to 312 days. In every case the foreign pigmentation faded out just as rapidly after this second inoculation from the same donor as it had done after the first inoculation.

(iv) *The immunity phenomenon*

Any pigmentation of foreign origin on any animal may at any stage be bleached out at will by grafting skin in adequate dosage from the animal whose cells initiated the pigmentation to the animal on which it has become established (plate III, figs. 21 and 22). All that this operation does is to bring about deliberately and under experimental control the bleaching process which in a certain proportion of animals has already been seen to occur "spontaneously." The process is distantly analagous to the cure of a bacterial or virus infection by immunological means: in the sense of this analogy, guinea-pigs are "cured" of an attack of foreign pigmentation. Immunisation may also be used prophylactically. If an orthodox skin graft is transplanted from one guinea-pig to another at the same time as a suspension of its epidermal cells is seeded on another area of the same recipient, pigmentation is bleached out almost immediately after its inception. The failure of orthodox skin homografts themselves to initiate pigment spread, and the regular failure of pigment spread in animals in which the process was initiated by too high a concentration of foreign cells, all point to foreign cell *dosage* as a factor critical for the successful initiation of pigment spread. It should be added that the guinea-pigs used throughout all the experiments recorded in this paper are of such genetic diversity that all orthodox skin homografts invariably submit to the immunity reaction they provoke and break down completely.* We have no record of any such graft surviving longer than 25 days. The fact that skin homografts break down no less promptly when transplanted to guinea-pigs already carrying widespread areas of pigmentation initiated by cells from the same donor shows that the recipients are not in any degree desensitized to foreign cells from the same source.

There is one highly qualified exception to the rule that an immunising graft from the same source will invariably bleach out pigmentation of foreign origin. This is when pigmentation initiated in a guinea-pig R by cells from a guinea-pig D is transferred back to a white area on D by a seeding operation in the reverse direction. Massive immunising grafts transplanted from R to D then fail to influence the pigmentation of D in any way. These experiments merely show that the foreign pigmentary matter on R, in whatever form it may be, retains the antigenic specificity of its original donor.

* Unpublished studies on skin homografts in adult mice by McDonald and Medawar show that the degree of genetic uniformity required before skin begins to survive homologous transplantation in certain combinations between mice is roughly that achieved by 10 successive generations of brother-sister mating.

Because of their distinctness and ease of handling, we normally use square black ear-skin Thiersch grafts (area 6-16 mm.^{2}) for immunisation, and transplant them in the orthodox way (Billingham and Medawar, 1948*a*). With one exception their survival time, as judged by outward inspection, has ranged from 8 to 18 days. Breakdown entails the disengagement and degeneration of the epidermis preceded and accompanied by a violent inflammatory reaction in the homograft dermis which is characteristically associated with a massive invasion of the dermal collagen by round cells and the stagnation and rupture of its blood and lymph vessels (plate IV, fig. 27). The bleaching of the foreign pigmentation elsewhere on the same animal is first perceptible when this reaction is at its height, and is associated with a round-cell infiltration of a much milder character (plate IV, figs. 28 and 29). Bleaching so induced (plate III, figs. 21 and 22) does not differ from that which in some animals takes place "spontaneously," and a steadily decreasing number of black hairs may survive the complete depigmentation of the surface epidermis by as long as 80 days.

Immunisation is systemic in effect: an immunising graft on one side of the chest will cause foreign pigmentation to disappear from the skin of the other side of the chest or from the sole of the foot. It had already been found that foreign pigmentation simultaneously induced in two different positions on the same animal—on both sides of the chest in one trial, and on the thigh and the nape of the neck in another—underwent a bleaching process of the same type and tempo in both positions.

Because of the very high degree of individual specificity shown by the homograft reaction (Medawar, 1946*a*), the use of an immunising graft from a donor *other* than that responsible for establishing the foreign pigmentation is theoretically unsound, and its results are unpredictable. A single experiment has shown that the antigenic specificity of the individual is no less conspicuous in seeding operations than in orthodox grafting. Four distinct areas on the chest of a single recipient were seeded with pigmented epidermal cells from four different donors, and pigment spread arose from each one. Bleaching began at 20, 30, 80 and 90 days respectively, after the establishment of correspondingly large areas of spread. In one area the persistence of pigmented hairs 120 days after the beginnings of bleaching prompted us to re-establish the surface pigmentation by the operation already described in section (ii). Active melanogenesis and pigment spread still continues at this one site.

Areas of induced foreign pigmentation have been removed at 12, 15, 16, 26, 42, 56 and 74 days after immunisation for orthodox histological examination (plate IV, figs. 28, 29 and 32) and for treatment by Gairns' gold-impregnation method for dendritic cells (Billingham, 1948)—*i.e.* after periods extending from just before the beginning of the induced bleaching to somewhat after its visible

completion. A normal complement of " white " dendritic cells has been revealed in each instance. It has, moreover, been shown on each of four occasions that a fully-bleached area of foreign pigmentation is " re-infectable " by pigmentation of native origin in the ordinary course of pigment spread. This has been demonstrated either by transplanting a small black ear-skin autograft to the centre of the bleached area, in which case pigment spreads progressively outwards, or by ringing the bleached area with black ear-skin autografts which in due course obliterate the bleached area enclosed within them (plate IV, fig. 30). There is therefore indirect evidence that the native dendritic cells of the artificially pigmented white skin are at least not wholly destroyed by the immunisation process, and that bleaching primarily involves the reconversion of pigmentary dendritic cells into their non-pigmentary analogues. Any more confident inference must be deferred until better histological methods have been devised for studying the dendritic cells of white skin. Orthodox histological study by microtome sections has proved to be totally inadequate.

It is a matter of some theoretical importance that bleaching by immunisation can be achieved not only by the transplantation from the original donor of black skin, which contains pigmentary dendritic cells, but also of white skin, which contains no pigmentary dendritic cells, and of tongue grafts, which contain no dendritic cells at all. Experiments making use of subcutaneous and intraperitoneal immunising grafts of spleen and lymph node, neither of which contains any epithelial ingredient, are not yet complete. This lack of tissue specificity conforms exactly with the immunological behaviour of ordinary skin homografts : a rabbit for example, may be immunised against skin homografts by an earlier intradermal injection of leucocytes from the same donor (Medawar, 1946*b*).

The purely immunological observations so far reported are equally consistent with two alternative interpretations of the nature of induced foreign pigmentation : (*a*) that the foreign pigmentation is maintained throughout by the survival, proliferation and outward migration of surviving foreign pigmentary dendritic cells, and (*b*) that though the foreign pigmentation is quite certainly initiated by surviving foreign cells from the donor, it is maintained by the propagated infection of native dendritic cells with an antigenically foreign cytoplasmic ingredient. The observations which follow go a little way towards resolving this antithesis.

If the continued melanogenic activity of well-established patches of foreign pigmentation is due to the survival of living donor cells, it is clear that they must enjoy an exemption from the homograft immunity reaction which is denied them when transplanted *in situ* in orthodox skin homografts (which invariably break down) and in that percentage of transplantations done by the seeding method in which pigmentation " spontaneously " fades out after a variable length of time. (In

operations of this second type, foreign mesenchymal cells and collagen fibres are not transplanted : the destruction of melanophores when transplanted in whole skin cannot therefore be a mere secondary consequence of the violent inflammatory changes that occur in the homograft dermis.) Yet the fact that *any* area of foreign pigmentation may be bleached out by active immunisation suggests that the hypothetically foreign melanophores of the pigmented area are *not* exempt.

This inconsistency is aggravated by the following fact : that the total quantity of viable foreign cells transplanted in an immunising operation which leads to bleaching of a large area of foreign pigmentation is *very much smaller than the total number of foreign cells which, according to the terms of the melanophore survival hypothesis, must be supposed to be already in residence*. A skin homograft 6 mm.^{2} in area or less will promptly bleach out a pigment patch 300 mm.^{2} in area or more. If this pigment patch contains one foreign melanophore to twenty native Malpighian cells of the epidermis, it follows that there are already more than twice as many foreign cells in residence as are provided in addition by the immunising graft. It can hardly be that the dosage of foreign cells in any area of pigment spread is always so adjusted that the addition of half as many again, or less, is just what is required to start a bleaching reaction.

According to the alternative hypothesis, there is a clear distinction between the wholly foreign pigmentary dendritic cells which start pigment spread and then break down, and native dendritic cells which, carrying an antigenically foreign cytoplasmic ingredient, are responsible for its maintenance. If this distinction is valid it should be possible so to adjust the dosage of an immunising graft that when the foreign cells in it are manifestly destroyed the cells of the patch of foreign pigmentation either survive unchanged or submit to a merely transient bleaching. Both possibilities have been realised, though only by luck, for in a planned experiment the dosage of an immunising graft would have to be adjusted in relation to a quite unknown quantity, the antigenic disparity between donor and recipient, and the small size of the guinea-pig makes it in any case difficult to work accurately with low-dosage ratios.

It is otherwise with the cow, in which low-dosage ratios are obviously easy to achieve. Pigment spread takes place in the spotted Friesian cow as it does in the guinea-pig (plate IV, fig. 33). In the course of skin-grafting operations on Friesian cattle, done with other ends in view, four black ear-skin homografts were reciprocally interchanged between a pair of young dizygotic twins, the grafts being transplanted to a white skin area on the outer aspect of the foreleg. Pigment spread of intense colour around these grafts began within a few weeks of transplantation and continues to the present time ; the grafts themselves, after prolonged chronic inflammatory changes associated with a " weak " homograft reaction, eventually broke down. At the stage

illustrated by plate IV, fig. 34, 69 days after transplantation, the resurfaced remains of the originally black homografts are dead white, though each is framed within a bold black ring of "infective" pigmentation. Grafts on several other cows underwent similar changes.

Guinea-pig 139, carrying a patch of surviving and normal foreign pigmentation, was immunised by a skin graft from the same donor 51 days after its inception. The immunising graft, of which all traces have long since disappeared, broke down about 25 days after its transplantation, and 10 days later the surface pigmentation of the foreign pigment patch began to weaken and disappear. The surface pigmentation never quite vanished: 150 days after immunisation 10 stout black hairs and some smutty traces of genuine epidermal pigmentation still survived. The pigmented area thereafter very slowly returned to normal and pigment is now spreading at the periphery of a 150 mm.^{2} area 400 days after its first inception.

It has already been argued that the "seeding" operation owes its success partly to the fact that the initial dosage of foreign cells is so small that the immunity they provoke in the course of their supposed destruction offers no more than a slight temporary setback to the progress of pigment spread. This interpretation is, of course, guesswork; but the fact that partial immunisation causes only partial and temporary bleaching is directly demonstrable. On two occasions immunising grafts were removed very shortly before their final destruction: one such graft, grossly œdematous and infiltrated by round cells, is illustrated by plate IV, fig. 27. In both cases the foreign pigmentation underwent a transient incomplete bleaching and then returned to normal. Spreading patches surviving to the present time are now of 330 and 250 days' standing respectively.

A pronounced "spontaneous" bleaching that proves to be incomplete and merely temporary is exactly similar in principle. One instance is so extreme as to deserve special mention. Guinea-pig 204 was subjected to a homograft seeding operation on the sole of its right foot (*cf.* plate IV, fig. 31). Weak diffuse pigmentation recorded at 26 and 33-day inspections proved to have become weaker by the 50th day, and nothing remained to be seen at inspection on the 70th and 120th days. A routine inspection at the 288th day, however, revealed a weak patch of pigmentation, and this has gained in colour and has been slowly spreading until the present time, 311 days after operation.

There are, then, definite grounds for making an immunological distinction between genuinely foreign melanophores and those which may be supposed merely to contain a foreign cytoplasmic element. The interpretation of our immunological data in the terms of an infection theory has, however, special difficulties of its own. These will be dealt with in section 4.

(v) *The serial propagation of pigmentary activity*

In theory, there is no reason why pigmentation induced in a second animal by cells from a first should not itself be transmitted to a third and then in turn to a fourth, and so on. Having overcome the technical difficulties of "splitting" and preparing epidermal cell suspensions from body skin, as this experiment requires, we have so far carried such serial propagations to the fourth stage in two lines and to a third stage in a third. (The fourth stage represents the fifth animal of the sequence, counting the original donor as one.) The time interval between successive propagations has so far ranged between 80 and 131 days; in future maintenance it will be not less than 100. At each stage we now propagate pigment from one donor to not less than four recipients, in order to insure against the dangers of technical or immunological failure; and as an additional safeguard against the latter, a particularly low dosage of foreign cells is transferred to only one of the smaller type of seeding bed (fig. 2) on each recipient.

4. A DISCUSSION OF THE "INFECTIVE" THEORY OF PIGMENT SPREAD

The majority of the observations recorded in this paper have made it possible to discriminate between two alternative and mutually inconsistent interpretations of pigment spread; those of melanophore migration and infective cellular transformation. The inadequacy of the former has already been made clear, and certain shortcomings of the infection hypothesis may now be dealt with.

Repeated attempts have been made, using a variety of techniques, to initiate pigment spread by extracts of pigmentary dendritic cells injected into or otherwise applied to white skin. All such attempts have so far failed. The reasons which we have already discussed in a more general context (1948*b*) may now be enlarged upon. A pigmentary dendritic cell can infect its non-pigmentary neighbours because, as a "cytocrine" cell whose normal activity leads to the inoculation of pigment granules into the Malpighian cells around it, it is specially adapted to perform just that sort of function. Moreover, when two dendritic cells enter into anastomosis, the cytoplasmic bridge between them is a cell process that may be as much as 2μ in diameter. The hypothetical infective agent might therefore be much larger than a virus-like particle—a "plastogene," in Darlington's terminology, though its infective properties require it to be provisionally classified as a provirus (Darlington and Mather, 1949). There are good grounds for supposing that the infective agent is associated with the melanin granules, for the researches of Herrmann and Boss (1945), Riley *et al.* (1949) and DuBuy *et. al.* (1949*a* and *b*) have shown that the complex of enzymes associated with the formation of melanin in the ciliary body and in transplantable melanomata is recoverable from the pigment granules themselves. (DuBuy *et. al.* have impressive

evidence that melanin granules are variant forms of mitochondria. See also Reyni, 1924.) The analogy between melanin granules, as the " elementary bodies " of an infective agent, and the Borrel bodies of fowl-pox virus or the Paschen bodies of vaccinia need hardly be pressed, and it is noteworthy that Sonneborn's kappa factor in *Paramecium aurelia* has proved to be of this order of size (*cf.* Preer, 1948). That the infective agent is highly complex cannot be doubted, since it carries the antigenic specificity of the individual and is destroyed by active immunisation with grafts of white skin as well as black. Its complexity is perhaps analogous to that of the Rous fowl sarcoma virus, which is known to contain an immunologically chicken-specific component (Amies and Carr, 1939) ; this type of association between viruses and the ingredients of normal cells is by no means rare (*cf.* the reviews of Pirie, 1946, and Kidd, 1946).

Another objection to the infection hypothesis turns upon its immunological behaviour when propagated from one individual to another : it appears to be non-antigenic, or only feebly antigenic, when residing in the dendritic cells of the host, but at the same time it is capable of being destroyed by an immunising graft of living foreign cells. Even this type of behaviour, however, is not without precedent. Although it is widely thought that viruses within cells are exempt from the action of circulating antibodies, Kidd (1942) has summarised evidence which shows that the cells of transplantable tumours may be freed from extrinsic (" passenger ") viruses by passage through specifically virus-immune hosts.

We have no explanation for the fact that, after natural or induced bleaching, foreign pigmentation endures longer in the hair follicles than in the superficial skin.

It has already been suggested that the slow rate of spread of red pigmentation into white skin may be due to the presence of a more dilute or attenuated pigmentary system in red skin than in black. The very slow rate of spread of black pigmentation into red skin might be due to an antagonism between two closely related cytoplasmic pigmentary systems, analogous to that between related viruses (Luria and Delbruck, 1942 ; Henle and Henle, 1945). (We are indebted to Dr G. Pontecorvo for suggesting this interpretation.) Both black and red pigmentary systems may be present in " infected " red dendritic cells : the former is " dominant " only in the sense that it is darker in colour and so takes the credit for the cell's phenotypic appearance. A red pigmentary system in a black dendritic cell would give no outward evidence of its presence.

5. SUMMARY

The phenomenon of pigment spread in spotted guinea-pigs has been the subject of a further investigation. Red pigmentation is found to spread into white skins more slowly than black pigmentation, and black pigmentation encroaches upon red skin more slowly still.

Different degrees of attenuation of the pigmentary factors, and competition between different but closely related pigmentary systems, may be responsible for these differences of rate. When red skin is transformed into black, the red dendritic cells are apparently transformed into black dendritic cells.

Pigmentation may be initiated in one guinea-pig by foreign pigmentary dendritic cells transplanted in very low dosage from another, and may thereafter be serially propagated from animal to animal. New techniques have been devised to bring this about: the "grafting" of dilute suspensions of epidermal cells in Ringer's solution to recipient areas cut to such a depth that the transplanted cells are given direct access to the bases of the hair follicles.

Once started, pigmentation of foreign origin increases in area by pigment spread, and in a high proportion of animals it may persist for long periods or perhaps indefinitely. Such pigmentation may at any time be bleached out by transplanting a small immunising graft from the guinea-pig whose cells initiated the pigmentation to the recipient in which it is spreading.

The quantitative and experimental analysis of these phenomena seems to show that pigment spread is caused by a serially propagable transformation of non-pigmentary into pigmentary dendritic cells by a cytoplasmic ingredient of the latter which is capable of behaving "infectively." The transformation has not yet been brought about by cell-free extracts, possibly because the infective agent is physically associated with the melanin granules themselves.

Expenses involved in this work have been partially defrayed by grants from the Birmingham branch of the British Empire Cancer Campaign and from the Department of Plastic Surgery, University of Oxford. We wish to thank Miss Jean Morpeth for her technical help at all stages.

6. REFERENCES

AMIES, C. R., AND CARR, J. G. 1939.
J. Path. Bact., *49*, 497.

BILLINGHAM, R. E. 1948.
J. Anat., Lond., *82*, 93.

BILLINGHAM, R. E. 1949.
J. Anat., Lond., *83*, 109.

BILLINGHAM, R. E., AND MEDAWAR, P. B. 1948*a*.
Heredity, *2*, 29.

BILLINGHAM, R. E., AND MEDAWAR, P. B. 1948*b*.
Brit. J. Cancer, *2*, 126.

DARLINGTON, C. D., AND MATHER, K. 1949.
The Elements of Genetics. London.

DUBUY, H. G., WOODS, M. W., BURK, D., AND LACKEY, M. D. 1949*a*.
J. Nat. Cancer Inst., *9*, 311.

DUBUY, H. G., WOODS, M. W., BURK, D., AND LACKEY, M. D. 1949*b*.
J. Nat. Cancer Inst., *9*, 325.

HENLE, G., AND HENLE, W. 1945.
Amer. J. med. Sci., *210*, 369.

HERRMANN, H., AND BOSS, M. B. 1945.
J. cell. comp. Physiol., *26*, 131.

KIDD, J. G. 1942.
J. exp. Med., *75*, 7.

KIDD, J. G. 1946.
Cold Spring Harbor Symp. Quant. Biol., *11*, 94.

LOEB, L. 1945.
The Biological Basis of Individuality. Springfield.

LURIA, S. E., AND DELBRUCK, M. 1942.
Arch. Biochem., *1*, 207.

MASSON, P. 1948.
Sp. Pub. N.Y. Acad. Sci., *4*, 15.

MEDAWAR, P. B. 1944.
J. Anat., Lond., *78*, 176.

MEDAWAR, P. B. 1945.
J. Anat., Lond., *79*, 157.

MEDAWAR, P. B. 1946*a*.
Brit. J. exp. Path., *27*, 9.

MEDAWAR, P. B. 1946*b*.
Brit. J. exp. Path., *27*, 15.

MEDAWAR, P. B. 1948*a*.
Brit. J. exp. Path., *29*, 58.

MEDAWAR, P. B. 1948*b*.
Quart. J. micr. Sci., *89*, 239.

PIRIE, N. W. 1946.
Cold Spring Harbor Symp. Quant. Biol., *11*, 184.

PREER, J. R. 1948.
Amer. Nat., *82*, 35.

REYNI, G. S. 1924.
J. Morphol., *39*, 415.

RILEY, V. T., HESSELBACH, M. L., FIALA, S., WOODS, M. W., AND BURK, D. 1949.
Science, *109*, 361.

RUSSELL, E. S. 1948.
Genetics, *33*, 228.

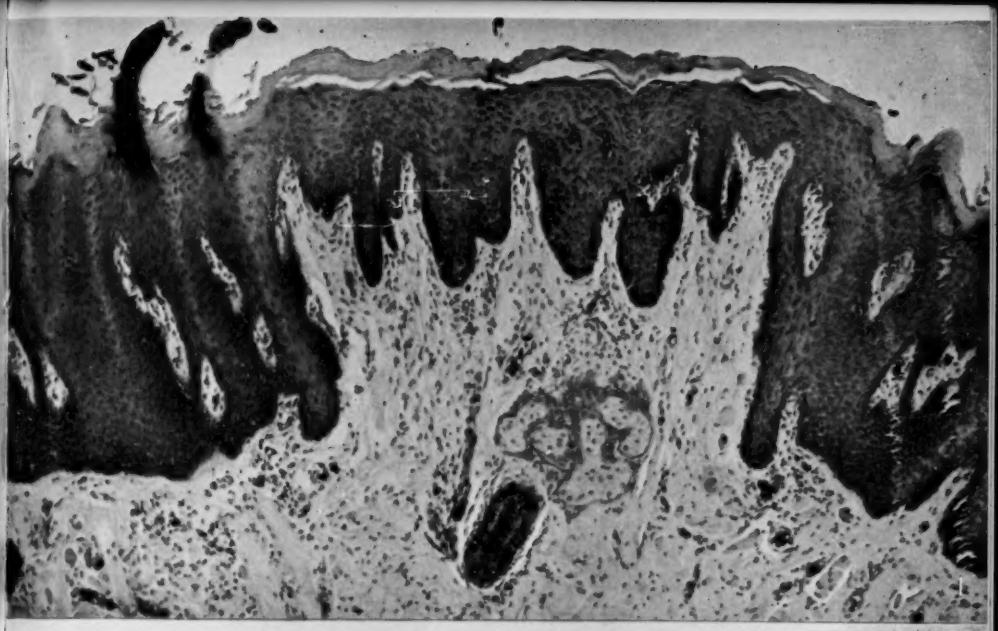
Plate I

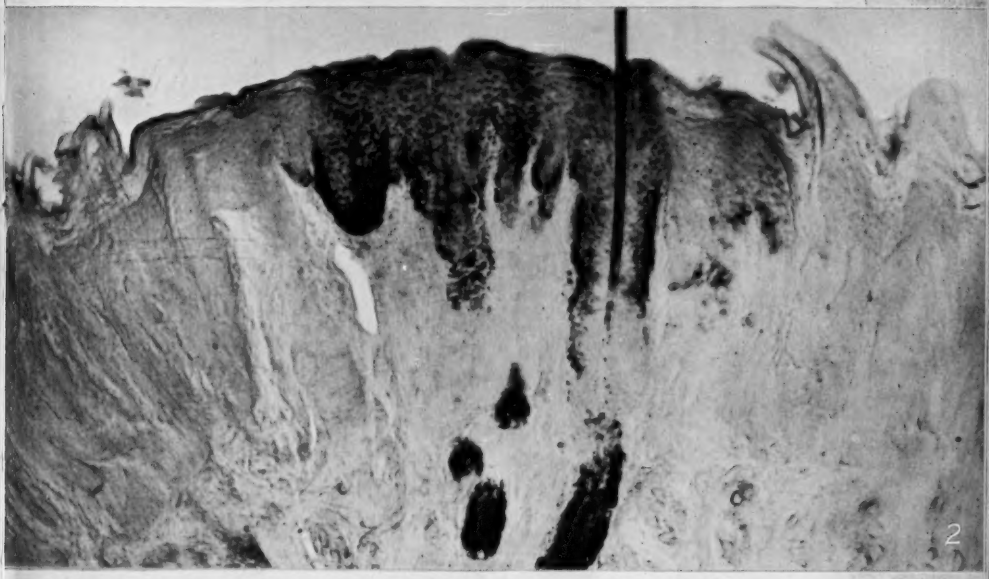
FIG. 1.—Vertical section through a black ear-skin graft 280 days after transplantation to the dorsum of the tongue: Ehrlich's hæmatoxylin and eosin. Note the form of the cuticle, the sebaceous glands, and other evidence that the ear skin epithelium has conserved its exact specificity of histological type. No pigment whatsoever has spread into the neighbouring tongue epidermis, from which dendritic cells are absent (*cf.* fig. 2). ×110.

FIG. 2.—See fig. 1: a vertical section at a different level, stained with carmalum alone. Note the hair follicles and the complete failure of pigment from the ear-skin graft to infect the neighbouring tongue epidermis. ×110.

FIG. 3.—Black (lower) and dark red (upper) ear-skin autografts 220 days after transplantation to white chest skin. Pigment spread has been much more rapid from the former (*cf.* figs. 4 and 5). ×1¾.

FIG. 4.—Black (lower) and dark red (upper) ear-skin autografts 232 days after transplantation to white chest skin. Pigment spread has been more rapid from the former (*cf.* figs. 3 and 5). ×1¾.





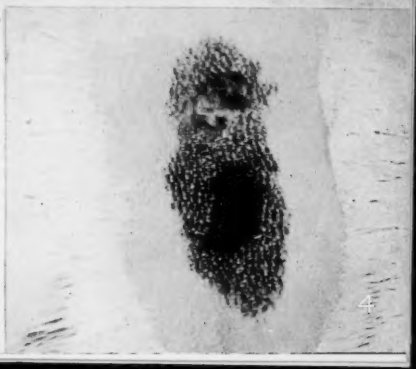


Plate II

FIG. 5.—A series of ear-skin autografts 555 days after transplantation to the chest. (*a*) An originally pale red graft lying within the black skin in the left half of the photograph, and now almost wholly encroached upon by black pigment. Only a very small irregular central patch remains light red in colour. (*b*) A black skin graft (lower right), pigment spread from which has met, but seems to be moving round and avoiding, the pale red pigment which has spread more slowly from (*c*) a pale red graft transplanted to white skin (upper right). Grafts (*a*) and (*c*) were originally of the same colour; now, after 555 days, grafts (*a*) and (*b*) are of the same colour. For views in higher power, see figs. 9-13. $\times 1\frac{3}{4}$.

FIG. 6.—A black ear-skin graft 301 days after transplantation to dark red chest skin. There has been no trace of pigment spread (*cf.* figs. 7 and 8). $\times 1\frac{3}{4}$.

FIG. 7.—Another black ear-skin graft 301 days after transplantation to red chest skin of medium colour. The edges of the graft are indistinct, but pigment spread is not yet visible (*cf.* figs. 6 and 8). $\times 1\frac{3}{4}$.

FIG. 8.—A black ear-skin graft 623 days after transplantation to pale red-coloured chest skin. The graft is surrounded by a feebly developed annulus of pigment spread (*cf.* figs. 6 and 7). $\times 1\frac{3}{4}$.

FIG. 9.—The epidermis of an originally pale red ear-skin graft 555 days after transplantation to black chest skin (see fig. 5). The epidermis is seen from the under side after weak Dopa treatment, and is taken from that part of the graft wholly encroached upon by black pigment. The epidermal pattern characteristic of ear skin has been fully conserved and a normal number of fully black melanophores may just be discerned (see figs. 10 and 11). $\times 120$.

FIG. 10.—A portion of fig. 9 seen in higher power (see also fig. 11) and showing the normal complement of fully black melanophores only, where formerly only red melanophores were present (figs. 12 and 13). $\times 300$.

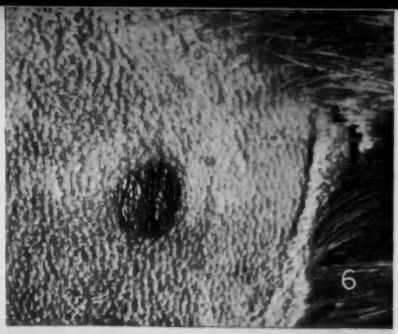
FIG. 11.—As fig. 10: another field. $\times 300$.

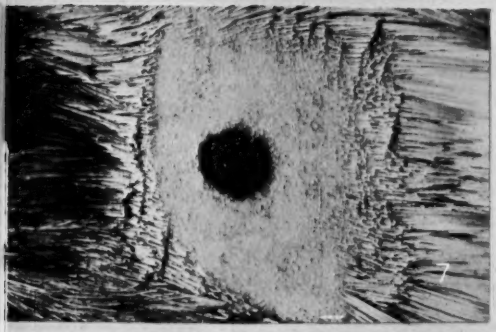
FIG. 12.—Red melanophores from the central "uninfected" patch of a pale red ear-skin graft 555 days after transplantation to black chest skin (see fig. 5). The processes of the red dendritic cells, almost impossible to discern in living skin, have been rendered visible by weak Dopa treatment. Contrast figs. 10 and 11, in which red melanophores are seen after transformation to the black type, and see fig. 13. $\times 300$.

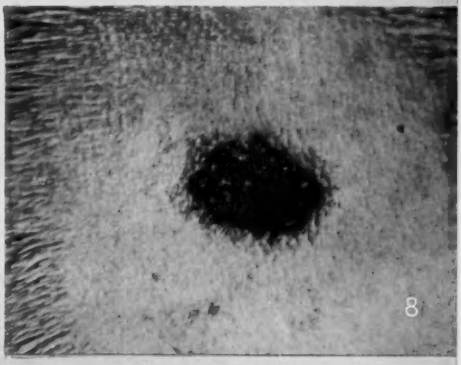
FIG. 13.—As fig. 12: another field. $\times 300$.

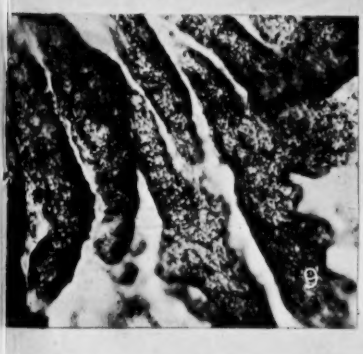
FIG. 14.—Illustrating the characteristics of pigment spread initiated by a seeding operation: a homograft seeding 127 days after its initiation by a very low dosage suspension in Ringer's solution of black ear-skin cells from another guinea-pig. No graft centre is visible. A sectional view of this graft is illustrated by fig. 17. $\times 3\frac{3}{4}$.

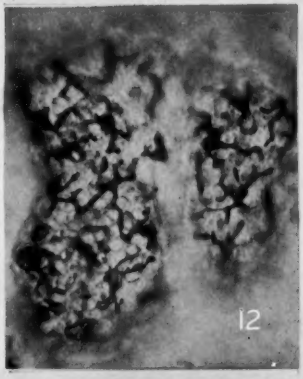
5

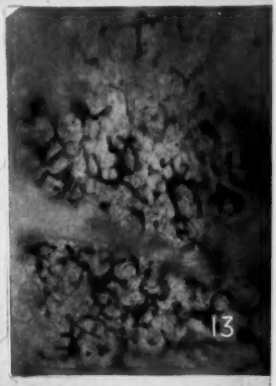

6


7


8


9


12


13

10

11

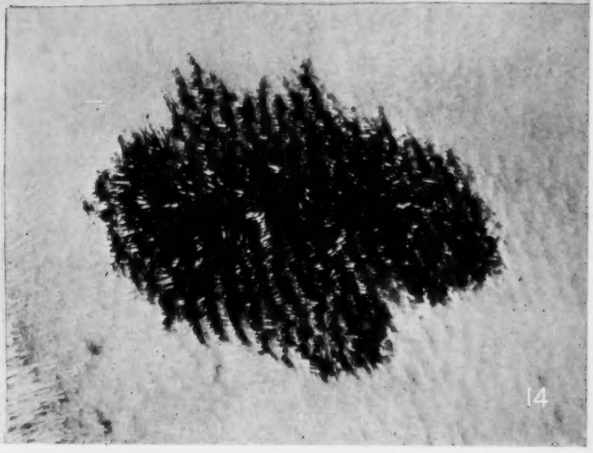

14

Plate III

FIG. 15.—Patches of native and of foreign pigmentation 86 days after their initiation by seeding operations making use of autologous and homologous cell suspensions respectively. The two are indistinguishable (*cf.* fig. 16). The pigment patches are shown in sectional view by figs. 18 and 19. ×2¼.

FIG. 16.—As fig. 15 : autograft and homograft seedings 86 days after their initiation in another guinea-pig. ×2¼.

FIG. 17.—A sectional view of the homograft seeding of 127 days' standing illustrated by fig. 14. The preparation can be distinguished from normal black chest skin only by a slight persistent hyperkeratosis. ×68.

FIG. 18 (compare fig. 19).—A vertical section through the perfectly normal homograft seeding of 86 days' standing illustrated by fig. 15. Although the mesenchyme cell population of the dermis is slightly denser than in normal skin, it is no more dense than in the control, fig. 19. ×68.

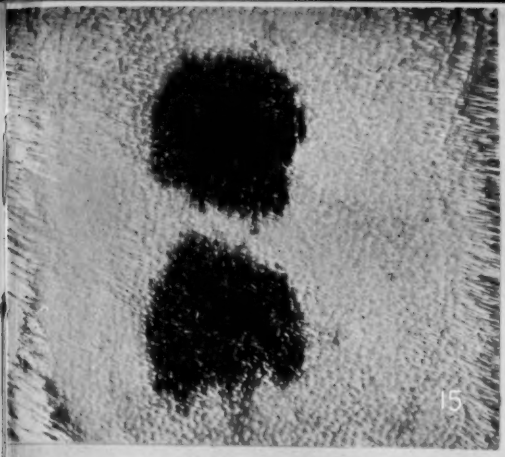
FIG. 19 (compare fig. 18).—A vertical section through the autograft seeding of 86 days' standing illustrated by fig. 15. Its condition is virtually identical with that of the homograft seeding, fig. 18. ×68.

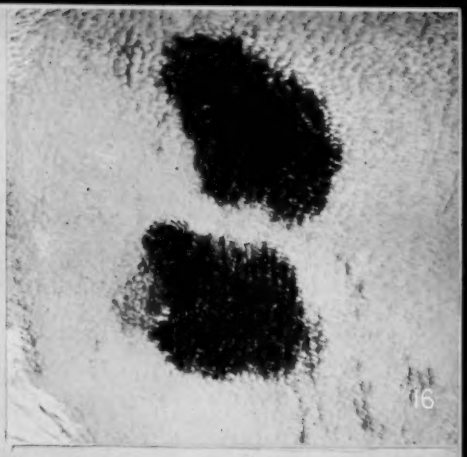
FIG. 20.—Illustrating the characteristics of an early stage of the bleaching process in induced pigmentation of foreign origin : a homograft seeding of 72 days' standing shown 26 days after the transplantation of an immunising graft from its original donor. The epithelium over the primary seeding centre has become smokily translucent and the peripheral pigmentation has weakened in colour and lost its crisply reticular pattern. ×2½.

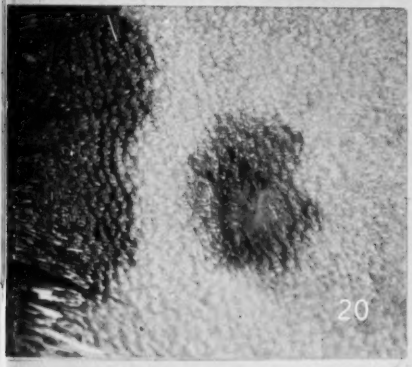
FIG. 21.—A perfectly normal homograft seeding of 50 days' standing, immediately before the transplantation of an immunising graft from the animal whose cells initiated the pigmentation. See fig. 22. ×1¾.

FIG. 22.—The homograft seeding illustrated by fig. 21, here seen 20 days after the transplantation of an immunising graft. Weak pigmentation is still visible in the primary seeding centres, but has almost disappeared from the peripheral area of pigment spread. ×1¾.

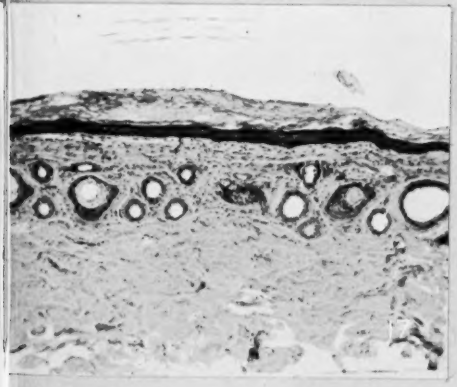
FIG. 23.—Illustrating the operation of " expansion " by which the total area of pigmentation of foreign origin may be rapidly raised. Two Thiersch grafts were cut from within the perimeter of the large primary homograft seeding (to the left) 146 days after its inception, and transplanted to the normal white skin on one side of it. The present photograph, taken 103 days later, shows spread from the secondary grafts about to coalesce with the primary seeding (*cf.* figs. 24 and 25). ×1½.

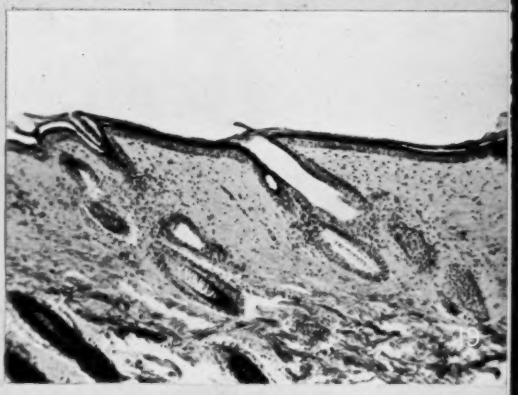

15


16

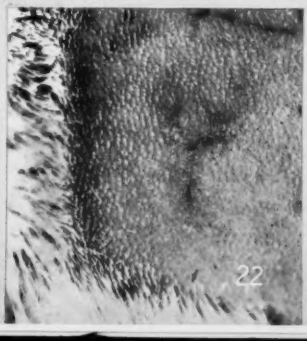

20

18





21


22

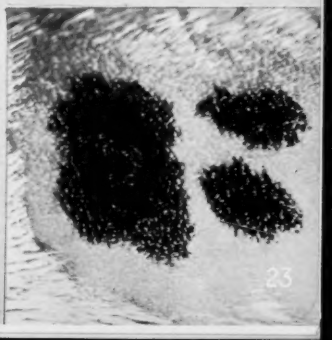

23

Plate IV

FIG. 24 (*cf.* fig. 23).—Showing the results of an expansion operation carried out in two stages. A Thiersch graft was cut from within the perimeter of the black primary seeding (shown uppermost) 164 days after its inception in the white chest skin of a spotted chocolate-and-white guinea-pig, and transplanted to the lowermost position. Fifty days later a second graft cut from within the primary seeding was fitted into the white skin still remaining between it and the pigment spreading from the first expansion graft. The photograph was taken 113 days after the second expansion, when the foreign pigmentation was of 327 days' standing in all. $\times 1\frac{3}{4}$.

FIG. 25.—Illustrating the size that a patch of pigmentation of foreign origin may reach by spread combined with expansion : shown 118 days after the expansion of a primary homograft seeding at 148 days.

FIG. 26.—Demonstrating that black pigmentation of foreign origin may spread in the white skin of a *red*-and-white guinea-pig. The foreign pigmentation is shown 267 days after its origin from a number of closely-spaced seeding beds of the smaller (" pinch graft ") type (see Text-fig. 2).

FIG. 27.—An orthodox black ear-skin homograft 16 days after transplantation as an immunising graft to a guinea-pig carrying a patch of pigmentation initiated by cells from the same donor 162 days beforehand. The graft dermis is œdematous and grossly infiltrated with round cells, but breakdown of the epidermis has not quite begun. The immunising graft having been removed, the homograft seeding (fig. 28) did not undergo permanent bleaching and is at present still normal 330 days from its inception. $\times 50$.

FIG. 28.—Biopsy specimen from the homograft seeding referred to in the legend to fig. 27, removed 16 days after the transplantation of an immunising graft. Compare the relatively mild lymphocytic reaction in the dermis with the violent reaction taking place simultaneously within the immunising graft (fig. 27). For a view in higher power, see fig. 29. $\times 50$.

FIG. 29.—A view in higher power of part of the biopsy specimen illustrated by fig. 28. $\times 250$.

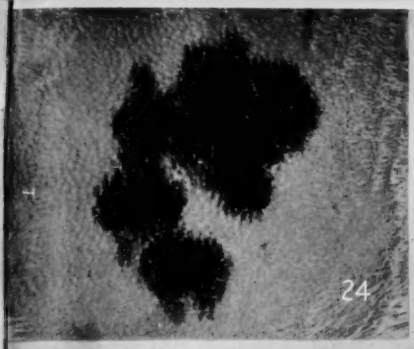
FIG. 30.—Showing that, after being completely bleached out by immunisation, the area formerly occupied by pigmentation of foreign origin is fully " reinfectable." The area was symmetrically ringed by nine small ear-skin autografts which 106 days later had completely obliterated the bleached skin within them by pigment spread. $\times 1\frac{3}{4}$.

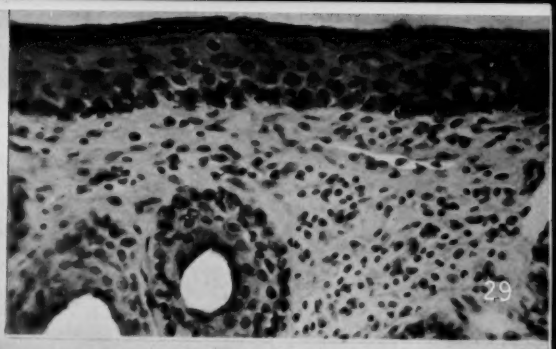
FIG. 31.—Pigmentation of foreign origin 162 days after its initiation by a seeding operation on the sole of the foot. $\times 2$.

FIG. 32.—Transverse section through a normal homograft seeding of 154 days' standing 26 days after the transplantation of immunising tongue grafts from the original donor. By this stage, pigmentation had been almost completely lost from the superficial epidermis. The inflammatory reaction is nevertheless of trivial intensity (*cf.* also figs. 27-29). $\times 68$.

FIG. 33.—White skin of the foreleg of a Friesian cow 153 days after the transplantation of a single large " pinch " graft from the black skin of the saddle region. The graft is symmetrically surrounded by a wide ring of typical pigment spread.

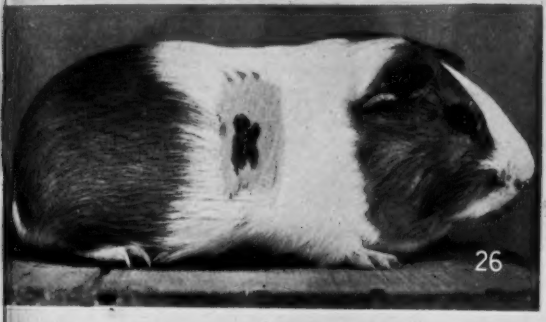
FIG. 34.—White skin of the foreleg of a Friesian cow 69 days after the transplantation of four black ear-skin homografts from its dizygotic twin. The annuli of pigment spread around the grafts have not been affected by the weak and prolonged immunity reaction, which has, however, led to the destruction of the homografts themselves, at this stage represented by white central patches.

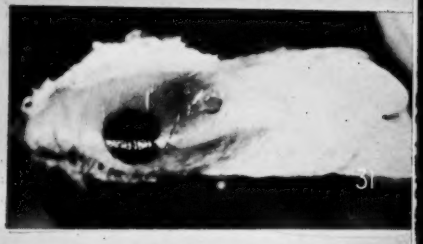

24

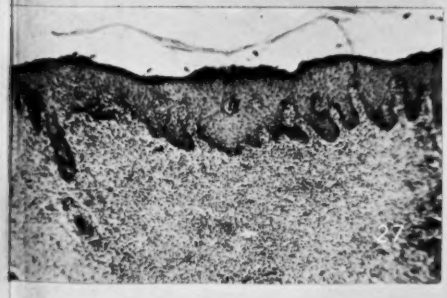

29

25

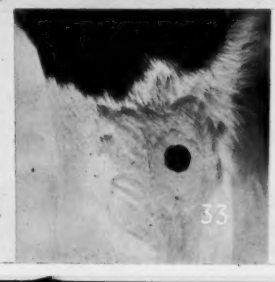
30

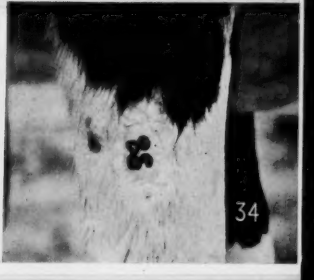

26


31


27

32


33


34

MUTATION OF THE KILLER CYTOPLASMIC FACTOR IN *PARAMECIUM AURELIA*

RUTH V. DIPPELL

Department of Zoology, Indiana University, Bloomington, Indiana *

Received 16.ii.50

SONNEBORN (1939, 1943) has shown that certain strains of *Paramecium aurelia*, known as "killers," liberate into the medium in which they live a poisonous substance called "paramecin." The latter has no effect upon the animals which produce it, but other strains of paramecia, known as "sensitives," are killed by its action. The killer trait was found by Sonneborn to be cytoplasmically controlled by "kappa," a self-multiplying, visible particle of which several hundred are present in the cytoplasm of each killer animal (Preer, 1946, 1948*b* ; Sonneborn, 1946). These bodies bear a striking resemblance to the nuclear genes in their ability to control an hereditary trait and in their power of self-duplication. However, one of the fundamental properties exhibited by nuclear genes, that of mutability, has, until the present study, been reported for cytoplasmic determinants of heredity in only two cases : mutation of plastids in higher plants (see review by Rhoades, 1946) and mutation of the genoid controlling CO_2 sensitivity in *Drosophila* (Goldstein, 1949). In 1948, this writer published a preliminary report of mutations of kappa. Sonneborn (1943) and Preer (1948*a*) had reported what they believed to be indications of a kappa mutation, but the cultures in question were never analysed. The present paper gives in detail the analysis demonstrating that mutation of kappa and not of the *K* gene provides the physical basis for five observed changes or mutations of the killer character. A brief account is included of the origins of the mutant stocks, the diverse types of killing they manifest, and the nature of the kappas present in the mutants.

I. THE MUTANT KILLER STOCKS

(*a*) *Detection and maintenance*

The paramecin produced by each killer stock causes sensitives to undergo a series of characteristic changes leading to death of the affected animals (Sonneborn, 1939, 1943, 1946 ; Preer, 1948*a*). The present paper deals with the paramecins produced by two killer stocks,

* Contribution No. 428 from the Department. Submitted to the Faculty of the Graduate School in partial fulfilment of the requirements for the degree Doctor of Philosophy in the Department. This investigation was supported by Indiana University and Rockefeller Foundation grants in aid of research on Protozoan Genetics at Indiana University.

The author was a Fellow of the American Association of University Women during part of the period over which this investigation extended.

47 and 51, of variety 4. The typical pre-lethal stages caused by the paramecin of killer stock 51 are shown in plate I, figs. (*a*)-(*e*). Stage (*a*) is recognisable about two hours after exposure and stage (*e*) about twelve hours after exposure of sensitives to stock 51 paramecin at 27° C. However, the time at which the first morphological changes become apparent and the duration of the various stages are dependent upon a number of factors, *e.g.* temperature and nutritive conditions of both killer and sensitive animals. The characteristic pre-lethal stage caused by killer stock 47 is development of a large vacuole in the posterior part of the body (plate I, fig. (*f*)).

Changes in the killer character of stocks 51 and 47 were regarded as mutations if they remained permanently heritable throughout vegetative and sexual reproduction. The mutants differed from the original killers either in the type of pre-lethal stages induced in sensitives or in the strength of killing as measured by the number of sensitive animals killed in a mixture with a given number of killers in a standard time interval.

Five different sensitive stocks were employed initially as detectors of the type of killing, but the most sensitive of these, stock 31 of variety 8, was used in most of the tests.

Whenever a culture displayed a mutant type of killing, some animals were individually isolated and grown at maximal rate, while others were isolated and grown at an average rate of one fission per day. The necessity for maintaining the isolations at different rates of growth is pointed out on p. 168. After each single animal isolation had given rise, by repeated fission, to a culture containing approximately 1000 animals, a sample of the culture was tested for its ability to kill and for its type of killing. Mutant killer clones thus obtained were carried as stock cultures, being given each day enough standard culture medium to permit an average growth rate of one fission per day. At approximately ten-day intervals new subcultures were started either as single animal isolations or as mass subcultures. Each new subculture was routinely checked for its killer character.

Successive mutants obtained from a given killer stock are designated as *m*1, *m*2, etc., following the stock number (47 or 51). In a preliminary paper (Dippell, 1948), the four mutants of stock 51 were referred to as the " first " to " fourth " mutants ; these correspond to the mutants 51*m*1 to 51*m*4 of the present paper, except that 51*m*1 and 51*m*2 are here reversed (*i.e.* the " first " mutant of the earlier paper is here designated 51*m*2). The change manifested by the culture designated as 51*m*4 proved transient ; as it was not a true mutation, 51*m*4 is not dealt with further in this paper.

(*b*) *Origin of the mutant stocks*

Of the five mutations analysed in this study, all arose spontaneously in so far as known.

Origin of 51*m*1 *and* 51*m*2.—Reserve cultures of each stock are

maintained in this laboratory in duplicate bottles fed once every three weeks. The duplicate bottles of stock 51 were subcultured from a common source in 1946 and in the following year one of these bottles was found to contain 51*m*1, the other, 51*m*2.

Origin of 51*m*3.—This mutant was found by Preer in a culture of stock 51 possessed by J. A. Harrison of Temple University and obtained originally from Sonneborn.

Origin of 51*m*5.—A single animal isolated from a typical culture of stock 51 produced descendants with markedly reduced killing power. One of the descendants was isolated and yielded the mutant culture 51*m*5.

Origin of 47*m*1.—In our laboratory, stock 47 lost the killer character during a period of several years when it was maintained in stock bottles but not employed for experiments. While it was still a killer, a sample of the stock was sent to Father J. A. Frisch of Canisius College. In 1946, he returned a subculture to Sonneborn's laboratory. This was immediately found to be characterised by a type of killing identical with that of typical stock 51. However, Father Frisch never possessed stock 51. The fact that 47*m*1 was derived from stock 47 and not from stock 51 was, moreover, demonstrated by breeding analysis (p. 173). (This same type of analysis showed that the remaining mutants were derived from stock 51.)

(c) *The phenotypes of the mutant stocks*

Among the five mutant killers, only two types of killing have thus far been found. The first type produces changes in sensitives which appear to be identical with those produced by 51 paramecin and will be referred to as 51 killing. The second type, discovered in 51*m*1, differs strikingly from 51 and partially resembles the killing action of stock *G* (variety 2) described by Sonneborn (1939) and more extensively by Preer (1948*a*). This type of killing is known as "spinner" or "Sp." Sensitive animals exposed to 51*m*1 paramecin reverse their normal direction of rotation and subsequently increase the speed of this rotation. As this is accompanied by little or no forward movement, it results in violent spinning of the sensitives on their longitudinal axes. In later stages, animals commonly develop one or two large vacuoles within the body, form along the aboral body surface a huge blister into which the body contents subsequently empty, or retain a somewhat normal appearance but settle to the bottom and become almost completely paralysed before death (plate I, figs. (*g*), (*h*) and (*i*)). This type of killing progresses to completion more rapidly than that of 51 or *G*.

Of the five mutants thus far analysed, one (51*m*1) produces only the Sp type of killing and one (51*m*5) produces only the 51 type of killing. The mutant stocks 51*m*2 and 51*m*3 are mixed with respect to the two types of killing: cultures derived from *single* animals of these mutant stocks kill some sensitives in one way and some animals of the same

sensitive stock in the other way. Mutant 47*m*1 was first believed to manifest only the 51 type of killing but, following several months of culture, a small amount of Sp killing was also detected.

Some of these mutants are also distinguished in other ways. Thus, 51*m*5 kills very weakly (see p. 171), often not at all, and mutants manifesting the Sp type of killing can retain this type of killing only when grown less rapidly than three fissions per day at 27° C.

Finally, the mutants are characterised by their resistance to the two types of paramecin : in brief, each mutant is resistant to the type or types of paramecin it produces. Thus, 51*m*1, which produces only Sp paramecin, is resistant to this type only and is sensitive to 51 paramecin. The reverse is true for 51*m*5. The remaining mutants, producing both types of paramecin, are resistant to both. In agreement with these relations, table 1 shows the complex results observed when mixtures are made between any two kinds of killers.

TABLE 1

Results obtained when living animals of stock 51, the mutant killer stocks and sensitive stock 31 are mixed together in all possible combinations of two each. — *indicates no killing,* 51 *indicates 51 type of killing,* Sp *indicates spinner type of killing, and* −51 *indicates* extremely *weak and sporadic 51 type of killing. Each record represents a composite result of six separate tests carried out within a six-month period.*

Living animals	Living animals						
	Original killer stock 51	Mutant killer stocks					Sensitive stock 31
		51*m*1	51*m*2	51*m*3	51*m*5	47*m*1	
51	—	51, Sp	Sp	Sp	—	Sp	51
51*m*1	51, Sp	—	−51	51	−51, Sp	51	Sp
51*m*2	Sp	−51	—	—	Sp	—	−51, Sp
51*m*3	Sp	51	—	—	Sp	—	51, Sp
51*m*5	—	−51, Sp	Sp	Sp	—	Sp	−51
47*m*1	Sp	51	—	—	Sp	—	51, Sp
31	51	Sp	−51, Sp	51, Sp	−51	51, Sp	—

Simpler and clearer results (table 2), in complete agreement with the preceding, are obtained by mixing breis of each kind of killer with animals of each kind. Animal-free brei was obtained by repeatedly forcing a concentrated culture of the animals whose killing action was to be studied through a 25 gauge hypodermic needle until no intact paramecia remained (Sonneborn, 1950*a*). Living animals to be tested for resistance or sensitivity to this killer brei were then added to a known volume of the brei. Vegetative animals used in the tests were standardised by growing them in test tubes at an approximate rate of one fission per day for five days prior to testing. Mixtures were observed frequently over a forty-eight hour period.

From the data in tables 1 and 2, one can determine for each stock its type of killing and its resistance or sensitivity to the paramecins

TABLE 2

The results obtained when living animals of stock 51, the mutant killer stocks and sensitive stock 31 are mixed in all combinations with breis obtained from those stocks. (Cf. *Table 1 legend for explanation of symbols.*) *Each record represents a composite of the results of three separate tests conducted within a four-month period.*

Brei	Living animals						
	Original killer stock 51	Mutant killer stocks					Sensitive stock 31
		51*m*1	51*m*2	51*m*3	51*m*5	47*m*1	
51	–	51	–	–	–	–	51
51*m*1	Sp	–	–	–	Sp	–	Sp
51*m*2	Sp	–51	–	–	Sp	–	–51, Sp
51*m*3	Sp	51	–	–	Sp	–	51, Sp
51*m*5	–	–51	–	–	–	–	–51
47*m*1	Sp	51	–	–	Sp	–	51, Sp
31	–	–	–	–	–	–	–

produced by other stocks. These three aspects of the killer character, when combined, comprise the killer phenotype of a stock and are summarised in table 3.

TABLE 3

The killer phenotype of the mutants and original 51 killer stock. This table shows the type or types of killing action on sensitive stock 31. The strength of the killing reaction is indicated by the number of (+) *symbols. Also shown is the resistance* (R) *or sensitivity* (S) *of a given stock to the two general types of paramecin found among the mutant killers, viz. 51 paramecin associated with 51 type of killing and Sp paramecin associated with spinner type of killing.*

Stocks	Type of killing action on sensitive stock 31		Reaction to paramecins	
	51	Sp	51	Sp
Mutants :—				
51*m*1 . . .	–	++++	S	R
51*m*2 . . .	– to < +	++++	R	R
51*m*3 . . .	+++	+	R	R
51*m*5 . . .	– to < +	–	R	S
47*m*1 . . .	++++	< +	R	R
Original 51 . .	++++	–	R	S

2. ANALYSIS OF THE KILLER MUTATIONS

Sonneborn has shown that inheritance of the stock 51 killer character depends upon two constitutional factors : a cytoplasmic factor, kappa, and certain genes. The principal gene, *K*, is essential

for the maintenance of kappa but cannot initiate its production. If either *K* or kappa or both are removed, the killer trait is quickly lost. Another gene, *s*, in the killer stock 51 is also involved, for when this is replaced by *S* from certain sensitive stocks, kappa may slowly disappear (Sonneborn, 1947*b*). In view of this knowledge, the change from the original to the mutant killer character could conceivably be due to mutation of *K* to *K'*, controlling some modification of kappa or paramecin ; mutation of one or more other loci that could affect kappa or paramecin ; or mutation of kappa itself. These three possibilities were investigated by means of the following experiments, the first three of which answer the question of the existence of a gene difference between the mutant and the original 51 stock with respect to control of the killer character.

(*a*) Materials

Special stocks used in the genetic analysis.—In addition to the mutant and original stocks already mentioned, two stocks experimentally obtained by Sonneborn were employed in the genetic analysis. One of these, the "51 sensitive" or "51S" stock, was genically identical with killer stock 51 but lacked kappa and so was sensitive to paramecins produced by 51 and by other killer stocks. The stock was obtained by freeing 51 killer animals of kappa, using the method of growth at 33·8° C. (Sonneborn, 1950*a*).

The second stock used in the analysis, stock 186, is highly isogenic with killer stock 51 but contains the recessive *k* gene from sensitive stock 29 of variety 4. This stock was obtained by crossing 51 killers to stock 29, inducing autogamy in the F_1 killer cultures, and backcrossing a homozygous recessive to the killers. These steps were repeated through seven successive backcross generations. Stock 186 is one of the recessives extracted after the seventh backcross.

(*b*) Methods

Crossing stocks.—The methods employed for making crosses and for avoiding both selfing and cytogamy (double self-fertilisation) were those set forth by Sonneborn (1950*a*).

Isolation of the F_1 generation.—Newly formed conjugant pairs were isolated separately into culture medium at 27° C. After the completion of conjugation, the two members of each conjugant pair were separately isolated and allowed to reproduce by fission, the resulting clones representing the F_1 generation. (Sensitive animals can successfully be crossed to killers because they are not affected by paramecin during the process of conjugation.)

Culture of the F_1.—As mentioned on p. 168, it was necessary to cultivate some of the mutant-type killers at a slow rate of growth in order to maintain their killer character, while still other mutant killers and the original stock 51 could be grown at maximal rate without loss of the killer phenotype. Two kinds of media were thus employed

in the culture of the stocks. One type (standard medium) supported a maximal rate of fission and consisted of baked head lettuce medium inoculated with *Aerobacter aerogenes* (Sonneborn and Dippell, 1946). Stocks 51 and 51*m*5 were cultured in this medium. The second type of culture fluid, maintaining slow growth, consisted of " exhausted " culture fluid supplemented with standard medium (Dippell's method : Sonneborn, 1950*a*). Mutant stocks 51*m*1, 51*m*2 and 51*m*3 were cultured in this fluid. Mutant 47*m*1 was at first cultured in standard medium ; later, however, it was found to lose one of its types of killing under these conditions and thereafter was cultivated in " exhausted " culture fluid.

Testing the F_1 for killing capacity.—To test each exconjugant clone for killing, approximately 100 animals of the clone were placed in each of three slide depressions. Approximately 100 animals of a killer culture of the same type as used in the cross were added to one depression, (*a*), and 300 animals of a sensitive culture were added to a second depression, (*b*). The third depression, (*c*), consisted only of the line being tested (control). If killing occurred in (*a*) but not in (*b*) or (*c*), the F_1 clone was sensitive. If killing occurred in (*b*) but not in (*a*) or (*c*), the F_1 clone was a killer. Autolethality (*i.e.* killing in (*a*), (*b*) and (*c*)) was not observed. Results of tests were recorded both at the end of twenty-four and forty-eight hours in experiments involving the 51, 51*m*5 and 47*m*1 killers and at the end of approximately seven and twenty-four hours in experiments involving the 51*m*1, 51*m*2 and 51*m*3 killers, since in the latter three stocks killing action proceeded to completion more rapidly than in the first three. These two sets of observations on each test were sufficient to show the type of killing (*i.e.* the characteristic morphological changes undergone by the sensitives) and to provide an indication of the strength of killing as measured by the numbers of sensitives killed in a standard time interval in relation to the number of killer animals present. All clones except those that proved to be sensitive to the killers used in the cross, were further tested by mixture with the original 51 killers. If such a mixture showed 51 type killing, the clone being tested was sensitive to 51 paramecin. Of course, cultures shown by the first test to be Sp mutant killers would also produce mutant killing in the mixtures with 51 killers.

Maintenance of the F_1.—The F_1 clones were perpetuated in daily isolation culture (Sonneborn, 1950*a*) in standard medium. Under these conditions, autogamy is long prevented, thus permitting the heterozygous genotype to persist. This method, which permits reproduction at maximal rate, was unsuitable for killers that had to be grown slowly in order to retain the killer trait. For them, the following method was employed. Instead of daily isolations, daily mass subcultures were made and the cultures were kept at 22° C. This proved to be the most effective procedure for keeping reproductive rate low and at the same time avoiding autogamy.

Obtaining the F_2 generation.—The F_2 generation was regularly obtained by inducing autogamy in F_1 clones according to the methods described by Sonneborn (1950*a*). Animals in the climax stage of autogamy, as shown by their appearance in living condition and as confirmed by staining parallel samples, were isolated singly and maintained at 27° C. in the same way as set forth on p. 170 for the culture of the F_1 clones.

Testing the F_2 clones.—When 1000 to 2000 animals were present in each culture, the clones were tested for killing in the same manner as the F_1 cultures. On the basis of the results obtained, a 15 to 20 per cent. sample of the total number of lines tested was selected to hold for retesting after an additional 10 to 15 fissions. A few clones were retested for a third and fourth time as a check on the adequacy of the first test. Since later tests invariably confirmed the results of the first test, the following account will give the results of the first test only.

(c) Experiment I

In this experiment, each mutant killer was mated with the 51 sensitive stock described on p. 170. This stock, it will be recalled, is genically identical with the killer stock 51 but lacks kappa. F_1 killers from such a cross therefore should give rise at autogamy to an F_2 generation in which 50 per cent. of the clones are homozygous for the original 51 killer gene, *K*, and 50 per cent. are homozygous for the killer gene in the mutant. If now, the difference between a mutant and the original killer stock is due to a mutation at the *K* locus, then the F_2 generation obtained from the F_1 killer should show segregation into two types of killers, mutant and original. On the other hand, if the mutant killers and original 51 killer are effectively alike in genes controlling the killer character, then no segregation should occur in the F_2, and all clones should remain mutant type killers.

As appears in table 4, the results obtained with each of the five mutants show that the mutant killer character is not due to a mutation at the *K* locus, for all F_2 killer clones showed exclusively the mutant type of killing in spite of the fact that half of them must have been homozygous for the original *K* gene.

It will be noted that fewer F_2 clones were tested than were obtained. The discrepancy in the first four crosses is due to (1) failure of some F_2 isolations to grow, (2) production of extremely weak clones which could not be adequately tested, and (3) the elimination of some clones which proved, by cytological check, to be of vegetative rather than autogamous origin.

The same applies in part to the fifth cross, but in this case there is an additional cause of the discrepancy. The cross of 47*m*1 × 51S, unlike the others, is between two stocks originating from diverse sources in nature. Such inter-stock crossing invariably yields high mortality among the F_2 clones in contrast to nearly 100 per cent.

viability of the F_2 generation obtained from crosses between individuals descended from a common ancestor. (The high percentage of F_2 mortality obtained when 47*m*1 was crossed to stock 51 and the absence

TABLE 4

Summary of the results of crossing mutant killers to the 51 sensitive stock. Symbols : K = *killer ;* MK = *mutant killer of type used in cross ;* Or.K = *original unmutated killer ;* S = *sensitive. Within each cross, approximately the same number of* F_2 *clones was obtained from each* F_1 *clone.*

Cross	F_1 : No. of clones used as source for F_2	F_2 : No. of clones obtained	F_2 : Results								
			Observed			Theoretical					
						No mutation at *K* locus			Mutation at *K* locus		
			MK	Or.K	S	MK	Or.K	S	MK	Or.K	S
51*m*1K × 51S	3	90	88	0	0	*88*	*0*	*0*	*44·0*	*44·0*	*0*
51*m*2K × 51S	7	103	102	0	0	*102*	*0*	*0*	*51·0*	*51·0*	*0*
51*m*3K × 51S	5	119	116	0	0	*116*	*0*	*0*	*58·0*	*58·0*	*0*
51*m*5K × 51S	6	90	89	0	0	*89*	*0*	*0*	*44·5*	*44·5*	*0*
47*m*1K × 51S	3	180	103	0	0	*103*	*0*	*0*	*51·5*	*51·5*	*0*

of such mortality when 47*m*1 was mated to stock 47, indicated clearly that the mutant could not have arisen from stock 51 but must have originated from stock 47, see p. 167.)

The question may be raised as to whether the results obtained in the F_2 of the above cross are comparable with those obtained in the other four matings when two cultures derived from the same stock were crossed. Sonneborn (1943) has shown, however, that the *K* gene in stock 47 is allelic to the *K* gene in stock 51 and capable of supporting the kappa and killer character of the latter. In view of this fact and the desirability of employing the same culture of stock 51 as the parent for all crosses to the mutant stocks, 47*m*1 was mated to the 51 stock rather than to the 47 stock.

Experiment 1 bears on three additional points. First, the observations show that the mutant killers contain a kappa comparable to the kappa of the original killer. The evidence here is parallel to that adduced by Sonneborn (1943, 1945) in the analysis of the original killer : the sensitive mate of a killer animal gives rise to a killer clone only when cytoplasm passes from the killer to the sensitive during conjugation. In each of the five crosses, there were selected for further study some pairs which did not exchange cytoplasm and some pairs which did. In each pair of the first group, one exconjugant produced only sensitive progeny ; while in each pair of the second group, both exconjugants produced killer progeny and of the mutant type. Hence,

mutant killing, like original type killing, depends on a cytoplasmic factor, kappa.

Second, as with the original killers, the genes of the mutant killers are unable to initiate production of kappa. This is shown by the fact that the sensitive mates in the first group described in the preceding paragraph yielded no killer progeny either in the F_1 or in their F_2 descendants obtained by autogamy.

Third, certain other observations, together with the demonstration that mutant killing depends upon kappa, indicate that the latter found in the mutant stocks was derived from the kappa of the original type killers. The only alternatives to this conclusion are *de novo* origin of the kappa of the mutant killers, and infection from an external source. *De novo* origin seems practically excluded by the fact that kappa has never arisen *de novo* in any of the many cultures of *KK* sensitives carried in the laboratory, although some have been under observation for twelve years. It can, of course, be objected that *de novo* origin is more probable in killers than in sensitives, but there are no observations that suggest this. As to the possibility of infection from a foreign source, the available evidence (Sonneborn, 1948) shows that infection can occur only in the presence of high concentrations of kappa (10^8 particles per c.c.), a condition that was never remotely approached, so far as known, in the history of the mutant killers. Moreover, the fact that *KK* sensitives have never spontaneously acquired kappa argues against infection as it does against *de novo* origin. It thus seems that the kappa of the mutant killers could hardly have arisen in any other way than by descent from the kappa of the ancestral original type of killer.

(*d*) *Experiment* 2

The genic identity between the mutants and original stock 51 with respect to control of the killer trait was considered in experiment 2 by arranging the crosses so as to follow the original 51-type killing rather than the mutant-type killing analysed in experiment 1. Stock 51 killers were crossed to mutant killers or to sensitives derived from mutant killers by freeing the latter of kappa in the manner mentioned on p. 179. Each sensitive obtained in this way thus retained all of the genes possessed by the mutant killer from which it was derived. Pairs showing no cytoplasmic exchange comprised the F_1 generation. The 51 killer member of each pair was used to provide an F_2 generation by autogamy.

If a gene, and not a kappa, difference determined the difference between the mutants and the original 51 killer, then that half of the F_2 homozygous for the killer gene of the mutant should produce mutant killing, and the remaining F_2, homozygous for the killer gene from stock 51, should continue to maintain 51 killing. On the other hand, if the gene controlling the killer characters of the mutants and

original stock 51 are effectively identical, then no segregation should occur in the F_2, and all of the F_2 clones derived from the F_1 51-type killer should show 51 type of killing.

The results are summarised in table 5. In the first two crosses, 51 killers were crossed to sensitives derived from killer mutants 51*m*2 and 51*m*5. In the case of 51*m*3, however, this mutant killer was crossed directly to 51 killers, due to loss of the derived 51*m*3 sensitive as a result of poor cultural conditions. Mutant killers 51*m*1 and 47*m*1 were also directly crossed to 51 killers, since, at this time, adequate experimental techniques had not been devised to free these mutants of kappa.

TABLE 5

Summary of results of crossing mutant sensitives and killers to original stock 51 killers (see table 4 for legend)

Cross	F_1 : No. of clones used as source for F_2	F_2 ; No. of clones obtained	F_2 : Results								
			Observed			Theoretical					
						No mutation at *K* locus			Mutation at *K* locus		
			MK	Or.K	S	MK	Or.K	S	MK	Or.K	S
51*m*2S × 51K	4	60	0	60	0	*0*	*60*	*0*	*30·0*	*30·0*	*0*
51*m*5S × 51K	6	90	0	89	0	*0*	*89*	*0*	*44·5*	*44·5*	*0*
51*m*1K × 51K	3	90	0	88	0	*0*	*88*	*0*	*44·0*	*44·0*	*0*
51*m*3K × 51K	2	90	0	89	0	*0*	*89*	*0*	*44·5*	*44·5*	*0*
47*m*1K × 51K	2	120	0	69	0	*0*	*69*	*0*	*34·5*	*34·5*	*0*

Whether or not the F_2 clones were derived from F_1 51-type killers of a mutant killer × 51 killer cross or from a similar F_1 obtained from a mutant sensitive × 51 killer cross made no difference in the analysis ; both types of crosses yielded strictly comparable results, as shown by the table. All F_2 cultures derived from F_1 51-type killers were themselves 51 killers, although 50 per cent. of the F_2 clones were homozygous for the killer gene from the mutant stock. The 51 killer character thus can be maintained by the killer gene of each mutant stock, in the absence of the *K* gene derived from the 51 killer parent.

Experiment 2 provided a second important result. In the 51*m*1K × 51K cross, pairs undergoing cytoplasmic exchange were obtained and, although these were not employed as source material for the F_2 generation recorded in table 5, they were retained in order to observe the effect of the " mixed " cytoplasm upon the killer phenotype of the resulting F_1 clones. Exconjugants from five such pairs were grown slowly and tested for type of killing after nine fissions. An equal number of controls showing no cytoplasmic exchange were

similarly treated. It was found that each exconjugant of those pairs showing no transfer of cytoplasm retained its own type of killing, but both exconjugant members of those pairs exhibiting cytoplasmic exchange became " mixed " with respect to type of killing action. Animals derived from such exconjugants were capable singly of producing two kinds of paramecin, 51 and 51*m*1. The F_2 obtained by autogamy in such " mixed " clones, continued to exhibit mixed killing, although all were 100 per cent. homozygous for *all* their genes. With respect to the alleles at the *K* locus, half were homozygous for the *K* gene from 51*m*1 and half were homozygous for the 51 *K* gene. This result not only shows that mixed killing occurs in entirely homozygous material, but suggests that the mutant killer probably contains mutant types of kappa capable of multiplying and maintaining their character even when associated with original kappa in the same animal. Similar experiments using the 51*m*2 and 51*m*5 mutants in crosses to the 51 killer were begun but not completed. The only tests carried out were on F_1 clones, at approximately the eighth fission ; an F_2 generation was not obtained. In so far as these experiments progressed, however, they yielded results comparable to those of the 51*m*1K × 51K cross.

(e) *Experiment 3*

The preceding experiments give no information as to whether the *K* gene is essential for the maintenance of the mutant killer traits, as it is for the original killer trait, since all of the stocks thus far employed have been homozygous for this gene. Conceivably, the kappas in the mutant killers might have become independent of nuclear gene control, resulting in the altered type of killing. Although improbable, the possibility was directly tested by breeding experiments replacing the *K* gene of the mutants with *k*, which, in homozygous condition, does not permit the original kappa to persist and multiply (Sonneborn, 1943).

The source of the *k* gene in the present experiment was stock 186 which is otherwise largely isogenic with stock 51 (see p. 170). Each of the five mutants was crossed to stock 186 and the F_1 killers were used to get F_2 generations by autogamy. Theoretically, half of the F_2 should be homozygous for *K* and should show the mutant killer character. The other half of the F_2 should be homozygous for *k* and should also yield mutant killer clones if the mutant killer trait is independent of the genes, but should yield sensitive clones if the mutant killer, like the original killer trait, depends on gene *K*.

The results, with two exceptions to be dealt with later, are summarised in table 6. In each of the five crosses, the F_2 generation obtained from F_1 killers by autogamy consisted of mutant killers and sensitives in the 1 : 1 ratio required for a single gene difference. Although the number of F_2 clones obtained from each F_1 culture

was small, the results (with the above exceptions) were in complete agreement with a 1 : 1 ratio. Hence, the mutant killer, like the original killer character, disappears in the absence of gene *K*.

TABLE 6

Summary of results of crossing mutant killers to the recessive kk *stock 186*

Parents	No. F1 K clones used to obtain F_2	No. F_2 clones obtained	Results of tests on F_2 clones			
			Observed		Theoretical	
			No. K	No. S	No. K	No. S
51*m*1K × 186	5	90	40	46	*43·0*	*43·0*
51*m*2K × 186	8	120	59	57	*58·0*	*58·0*
51*m*3K × 186	4	118	63	54	*58·5*	*58·5*
51*m*5K × 186	3	90	43	47	*45·0*	*45·0*
47*m*1K × 186	3	180	48	43	*45·5*	*45·5*
Total . .	23	598	253	247	*250·0*	*250·0*

Additional confirmation was obtained by other crosses. Thus, three of the F_2 killers from the cross involving 51*m*5 were backcrossed to stock 186, and autogamy was induced in the backcross killers. These exautogamous clones included 73 killers and 80 sensitives, in agreement with the expected 1 : 1 ratio. Further, 6 of the F_2 sensitives from the same cross (51*m*5 × 186) were backcrossed to 51*m*5 and autogamy was again induced. The exautogamous progeny included 66 killers and 71 sensitives.

As mentioned above, two groups of F_2 clones were omitted from table 6 because the ratios obtained differed from all the others. The first was a group of 15 clones obtained from one F_1 culture from the 51*m*1 × 186 cross; this group included 14 killers and 1 sensitive. The probability of such a deviation from 1 : 1 is about 0·001. Such a result could be formally explained as due to the occurrence of macronuclear regeneration (Sonneborn, 1940) at the autogamy giving rise to this F_2 group, but such an explanation seems unlikely, for macronuclear regeneration is rare under conditions of these experiments. Since the F_1 culture was discarded before the aberrant F_2 results appeared, it was impossible to analyse the matter further, and no satisfactory explanation can be given. However, the F_1 culture from the other exconjugant of the same pair was also a killer and, fortunately, was retained. Therefore, this culture and another F_1 killer culture (which gave a normal ratio in the F_2) were backcrossed to stock 186. The two members of each backcross pair were separated at completion of conjugation, grown and tested before the occurrence of autogamy.

In such backcrosses, if no cytoplasmic exchange occurs between

mates, half of the total number of pairs should yield one sensitive and one killer member in each pair, and the remaining half should show both members to be sensitive.

In each of the two backcrosses, 16 pairs were obtained which showed no exchange of cytoplasm between mates. One backcross group yielded 7 killer-sensitive pairs and 9 sensitive-sensitive pairs; the other produced 6 killer-sensitive pairs and 9 sensitive-sensitive pairs (1 pair was lost). Although these groups are small, the results are in close agreement with the expected 1 : 1 ratio. Thus, the sister culture of the one that had given the aberrant F_2 ratio, did not itself give any aberrancy, either in the original F_2 or in the backcross, the total for the two diverse segregations being 18 : 13. The one member of this F_1 pair hence was clearly heterozygous, whatever may have been the peculiarity of the other member of the pair.

The second exceptional ratio was obtained in the cross of 47*m*1 × 186. From one F_1 clone, 26 F_2 killers and 9 F_2 sensitives were obtained by autogamy. The probability of such a deviation from 1 : 1 is about 0·006. As in the first exception, no explanation can be given, and again, since the F_1 was discarded after obtaining the F_2 isolations and before testing of the latter, the cross could not be repeated with the same material. Although the F_2 mortality was high (as is to be expected from this cross, see p. 173), the discrepancy could not be due to differential mortality, since other F_2 groups from the same cross yielded normal 1 : 1 ratios in spite of a similarly high incidence of death. Further evidence that normal segregation ratios ordinarily result from this type of cross was obtained by backcrossing one of the F_2 killers from a normal group to the recessive parent stock 186 and obtaining from the backcross a new generation by autogamy. This generation included 54 killers and 50 sensitives.

Although the two exceptional results remain unexplained, it is clear that ordinarily the data agree closely with the ratio required for segregation of a single pair of alleles and thus leave no doubt either of the dependence of the kappas in the mutants on gene *K*, or of the fact that mutant killers differ from stock 186 at only one effective locus, the *K* locus, just as does the original killer stock 51.

Summarising the results of the genetic analysis, it has been shown by experiments 1 and 2 that the killer mutations cannot be due to a mutation at the *K* locus. Further, the new characters cannot be the result of mutation at any other locus, for this would also have been detected by F_2 segregation in experiments 1 and 2 (which did not take place) and by more complex ratios in experiment 3 (which were not obtained). The two exceptions found in experiment 3 could scarcely be considered support for another interpretation in view of all the contrary evidence. We are thus forced to seek the basis of the mutant killer traits in the cytoplasm of the killer cell, mutation of kappa having already been indicated by these experiments as the most probable explanation for the altered killer character.

(*f*) *Experiment 4*

If different kinds of killing are due to different kinds of kappa, then mutants that show two kinds of killing must contain two kinds of kappa. This crucial point can be put to a decisive test by applying to such killers techniques that reduce kappa concentration until some or most of the cells contain only one particle, then altering the conditions so that the residual particle multiplies and restores the full complement (Preer, 1946). These procedures, in effect, yield (in a killer animal and its descendants) a pure culture of kappa arising from a single particle. They thus make it possible to discover whether pure cultures of kappa derived from different kappa particles of one mixed killer animal may be of two diverse types. If so, then some of the resulting killer clones should be pure for one kind of killing and some for the other kind manifested by the ancestral mixed killer. On the other hand, if the two types of killing by such mixed killers are not due to two types of kappa, then each and every pure culture of kappa obtained by the dilution and restitution technique should again control both types of killing characteristic of the mixed killer ancestor.

The methods applicable to the original 51 killer were developed by Sonneborn (1946, 1950*a*). Animals are exposed to a temperature (33·8° C.) at which 51 kappa cannot increase, although the paramecia multiply rapidly. This approximately halves the amount of kappa per cell at each fission. After a number of fissions (depending on the original kappa concentration) an appreciable fraction of the cells have no kappa and most of the remainder have a single particle. At this stage, the animals are isolated and placed at 27° C. with a limited amount of food daily (to keep fission rate at about one fission per day). Under these conditions, kappa multiplies much more rapidly than the paramecia, and those animals that retain one or more particles of kappa restore the full complement in the course of several days.

Specifically, these methods were applied by isolating vegetative killer animals singly into standard culture medium contained in test tubes in a 33·8° C. water bath. At this temperature, animals multiply regularly and at maximal rate for eight or nine fissions, after which division tends to become progressively slower and irregular. At the ninth fission, the culture contained in each tube was thoroughly mixed, and approximately one-half of the total number of animals was removed by withdrawing half of the total volume of fluid. This volume was then replaced by an equivalent amount of fresh culture medium preheated to 33·8° C. Volumes withdrawn were distributed to depression slides and animals contained in them were counted, singly isolated, grown at 27° C. and tested.

The result of applying this method to mutant 51*m*2 fully confirmed the existence, in this killer, of two distinct types of kappa associated

with two distinct types of killing. Of 193 animals isolated from 33·8° C. at the ninth fission, 30 produced cultures that were pure for Sp killing and resistance, but were sensitive to 51 killers (in the latter respect differing from the 51*m*2 parent), and 81 produced cultures that were non-killers but were resistant to 51 killers and sensitive to Sp killers (in the latter respect also differing from the 51*m*2 mutant). Thus, two distinct types were isolated, each differing from 51*m*2 : one type of culture was pure for a kappa controlling Sp killing and resistance ; the other was pure for a kappa controlling resistance to 51, but little or no killing. The combination of these two kinds of kappa would indeed account for the phenotype of the 51*m*2 stock : resistance to both 51 and Sp killing and production of Sp paramecin.

As was to be expected, some of the isolates in this series yielded cultures exactly like the original 51*m*2 killer. These would arise from animals containing at least one particle of each kind of kappa at the time they were removed from the high temperature. The frequency with which these occurred (67) is in reasonable agreement with calculations based on the assumption of random segregation of the two kinds of kappa particles.

The results obtained with a second mixed killer, 51*m*3, are in qualitative agreement with the results on 51*m*2. Of 271 animals isolated at the ninth fission, 36 died, 23 were sensitive to both 51 and Sp paramecin, 92 were 51 killers and sensitive to Sp paramecin, 74 were Sp killers and sensitive to 51 paramecin, and 46 were mixed killers like 51*m*3. Although the relative frequencies of these diverse classes are not in good agreement with the assumption of random segregation of two kinds of kappa, there can be no question as to the agreement with the main assumption of two distinct and separable types of kappa in the 51*m*3 mutant. One type of kappa controls 51-type killing ; when it is the only kind of kappa present, the animals are sensitive to Sp paramecin. The other type of kappa resembles one of those extracted from 51*m*2 : it controls Sp killing and when it is the only kind of kappa present, the animals are sensitive to 51 paramecin. When both kinds of kappa coexist in large quantities in the same animal, the latter kills in both ways and is resistant to both kinds of paramecin.

In contrast to the results on the mutant killers 51*m*2 and 51*m*3, the same methods applied to killers manifesting but a single type of killing (51, 51*m*1 and 51*m*5), yielded but a single type of killer culture in each case : all of the isolates obtained from stock 51 contained kappa controlling exclusively 51-type killing ; all of the isolates from 51*m*1 possessed kappa determining only the Sp type of killing ; and all of the isolates derived from 51*m*5 contained kappa controlling exclusively the 51*m*5 phenotype.

Altogether, there thus seem to be but three clearly distinguishable types of kappa in the five mutants and the original 51 killer stock.

One kind, 51 kappa, controls the original 51 type of killing, and can multiply as rapidly as the cells that contain it. The second kind, Sp kappa, controls the spinner type of killing, and multiplies more slowly than 51 kappa. The third kind, 51*r* kappa, controls extremely weak 51 killing, or none at all, but it confers resistance to 51 paramecin and is able to multiply as rapidly as 51 kappa. Preliminary cytological studies indicate other characteristics of these kappas : the Sp kappa, with the possible exception of this type in 47*m*1, seems to attain much higher concentration in the cell than does either 51 or 51*r* kappa ; and the frequency of double or multiple kappa particles may differ for different kinds of kappa.

Each of the diverse killers is characterised by the possession of one or two of these three kinds of kappa. The original 51 killer has 51 kappa only, 51*m*1 has Sp kappa only, and 51*m*5 has 51*r* only ; 51*m*2 has Sp *and* 51*r* ; 51*m*3 has Sp *and* 51. As set forth above in the discussion of experiment 2 (p. 175), it is possible to construct mixed mutants from cultures each of which possesses only one type of kappa. Thus a culture resembling 51*m*2 was constructed by crossing 51*m*1 to 51*m*5 and selecting conjugant pairs that exchanged cytoplasm. In the same way, a culture resembling 51*m*3 was constructed by crossing 51*m*1 to the original 51 killer.

These constructed killers further simulated the spontaneous mixed mutants in that the two kinds of kappa remained distinct and separable in them. Constructed types were cultivated for ten to twelve days and then subjected to the same procedure described above for isolating two kinds of kappa from 51*m*2 and 51*m*3. In each case, the two kinds of kappa that had been experimentally brought together were again experimentally separated from one another. The two kinds of kappa thus maintained their integrity even when in the same cell : each reproduced true to its own type, unaltered by the presence of the other.

Evidences previously cited (p. 174) led to the rejection of the possibilities that the kappas in the mutant stocks might have arisen *de novo* or by infection from an outside source. It was therefore concluded that these kappas were derived from the kappas of the two original killers, stocks 47 and 51. Among the mutant stocks there now exists at least two kinds of kappa that differ from both of the original kappas and that reproduce true to their new types. Each type controls a distinct kind of killing action. In sum, therefore, the physical basis of the killer character mutations has been found to lie in the mutation of kappa.

3. DISCUSSION

(1) *The nature of kappa mutations.*—The nature of the kappa mutations reported in this investigation is still largely a matter of speculation. If the specificity of kappa is due to one or more haptene-like groupings in the kappa particle, then a mutation might result from a change or loss of one or more of such groupings. Another

possibility is suggested by the fact that in at least two of the five mutants examined, the original type of kappa is absent and *two* mutant types of kappa coexist. Since mutations of the killer character are detected very rarely, Sonneborn (1950*b*) points out that the existence of even one double mutant among the five examined could hardly be coincidental. Since at least two of the five mutants analysed are double mutants and since Preer's (1950) data show that the kappa particles of his stock *G* mutant killer is approximately one-half the size of that in the original *G* killer, Sonneborn suggests that double mutations might arise as a result of a splitting of kappa into two different parts. There is as yet, however, no critical evidence from the present data which bears on this point.

(2) *Kappa populations: equilibria and competition and their relation to the observed kappa conditions in the mutant killers.*—Three of the five mutant killers have maintained two kinds of kappa, presumably in all of their descendants, for a period of at least two years. This observation immediately suggests that some sort of population equilibrium has been attained between various combinations of two kinds of kappa. The conclusion, however, is not necessarily justified, for there is another mechanism that could lead to the same result: viz. the mere coexistence of these two types of kappa within the cells could automatically perpetuate the combination by killing off all cells that lose either one of the two types of kappa. This would result in the appearance of typically affected animals within the culture. A simple way to obtain evidence as to whether maintenance of a mixed kappa population is due to such selective elimination of those animals that lose either kappa type, is to examine the culture for animals manifesting a response typical of sensitivity to either of the two kinds of paramecin in the culture. While it is often difficult to discover such sensitives in large mass cultures, their occurrence can readily be detected in small slide cultures of animals in nearly-starved condition.

Observations of this sort were carried out on two of the double mutants. In stock 51*m*3, animals were isolated singly into depression slides and grown at the rate of one fission per day until approximately 2000 animals were produced. At this stage, the animals are in proper nutritive condition to respond to paramecin action, and the presence of sensitive animals of either sort would be clearly manifested. Such observations were carried out on approximately 2000 descendants of each of 323 animals isolated from 11 different 51*m*3 subcultures at various times. Among the more than 600,000 individuals examined, not one sensitive animal of either kind was found. As a check, all of the 323 cultures were then mixed with known sensitive animals and were shown to be typical 51*m*3 killers, *i.e.* producing both 51 and Sp paramecins. This failure on the part of 51*m*3 clones to yield sensitive animals indicates that the combination of two kinds of kappa is not maintained, under such conditions, by selective elimination of animals that lose one kind of kappa. It would therefore seem that

the two kinds of kappa are not subject to competitive replacement, but are maintained as a natural equilibrium of the two kappa populations.

The situation is somewhat different in the mutant 51*m*2, which carries Sp and 51*r* kappas: in this case, any animal that lost Sp kappa would be killed off by those that retained it, but any animal that lost 51*r* kappa would probably survive, since the mixed killers (containing both Sp and 51*r* kappas) produce little or no 51 paramecin. In this case, therefore, selective elimination could operate to destroy animals that had lost Sp kappa, but it would not operate effectively to destroy those that had lost 51*r* kappa. The presence of the latter type of animal in cultures of 51*m*2 can be readily detected, however, by exposing these cultures to paramecin produced by the original 51 killer, for loss of 51*r* kappa makes the animals sensitive to 51 paramecin. This test was applied to some of the progeny of each of 211 single animal isolations from 51*m*2 clones. In no case were animals found that were sensitive to 51 paramecin; hence, no animals lacking 51*r* kappa were present in the cultures of 51*m*2. Parts of these same cultures were also tested by mixing them with known sensitives; the tests showed that all of the 211 isolates produced Sp killing. Thus, like 51*m*3, the 51*m*2 mutant maintains a population equilibrium of two kinds of kappa under the conditions of culture employed.

The third possible combination of kappas, 51 and 51*r*, has not been analysed, since it has never been found as a spontaneous mutant. On the other hand, there are several lines of evidence indicating that such a kappa combination might behave differently from the combinations that have previously been examined. First, as has been stated, these two kappas are the most nearly identical of those studied; yet, 51*r* is less active than 51 kappa and may utilise more effectively the intracellular material required for its maintenance and reproduction. This could give 51*r* a selective advantage in competition with 51 kappa. Secondly, 51*r* kappa is known to have arisen from 51 kappa and established itself free from the latter in 51*m*5, a fact difficult to explain unless the 51*r* kappa has the assumed selective advantage. Finally, the 51*m*2 mutant contains Sp and 51*r* kappas in equilibrium, without any 51 kappa being present. The absence of 51 kappa is the more remarkable since it is known from the condition in 51*m*3 that Sp and 51 kappas can establish an equilibrium. Hence, it would seem that the loss of 51 kappa from 51*m*2 is also to be interpreted as due to the selective advantage of 51*r* which displaced it. Taken together, these three lines of evidence warrant the tentative conclusion that 51 kappa is lost when 51*r* kappa is present. This conclusion can, of course, be tested by crossing 51 killers to 51*m*5 killers and obtaining cytoplasmic exchange between mates. This cross was actually made (p. 176), but the experiment was by no means conclusive since the mixed clones were followed for only eight fissions.

On the basis of the known equilibria established by 51 and Sp and by 51*r* and Sp, and on the indicated replacement of 51 by 51*r*

kappa when these two occur in the same cell, establishment of the mutants 51*m*2, 51*m*3 and 51*m*5 can readily be explained. The condition found in 51*m*3 (coexistence of 51 and Sp kappas) would arise if a particle of 51 kappa mutated to Sp kappa, for an equilibrium would eventually be established between these two kappas, and the remaining original 51 killers in the culture would be eliminated through the action of the Sp paramecin liberated by the mutants. Thus, the culture in which 51*m*3 arose would transform *in toto* to a mutant killer culture. The condition found in 51*m*2, coexistence of 51*r* and Sp kappas in the absence of 51 kappa, presents more of a problem. Here, however, after the origin of 51*r* kappa, 51 kappa would be expected to disappear. The Sp kappa present in the 51*m*2 mutant could have arisen before or after the origin of 51*r* kappa, or (on Sonneborn's hypothesis) at the same time. Once the mutant cell containing the kappa combination was established, the remaining 51 killers in the culture (as well as any cells which might have contained both 51 and 51*r* kappas or 51*r* alone) would have been eliminated by the action of Sp paramecin. Consequently, as in the case of 51*m*3, the entire culture would eventually contain only 51*m*2 animals. These conditions were, in fact, precisely those under which the 51*m*2 and 51*m*3 mutants were found.

Establishment of the 51*m*5 mutant is explained by a different set of conditions. Displacement of 51 kappa by 51*r* kappa has already been indicated. Thus, origin of 51*r* kappa within a 51 killer animal would result in the loss of the original kappa and production of a 51*m*5 animal. Both 51 and 51*m*5 animals would be expected, however, to survive equally well within the same culture, since Sp paramecin is absent and since either type of animal is resistant to the paramecin of the other type. Such a culture would appear as a " weak " killer culture, however, since 51*m*5 animals produce little or no paramecin. Single animal isolations made from a weak killer culture of this sort would thus be expected to include some 51*m*5 animals. It was under exactly these conditions that the mutant was found.

At present, it is not possible to give a satisfactory explanation of mutant 51*m*1 which contains only Sp kappa. There is no evident reason why Sp kappa should displace the 51 kappa from which it arose, since Sp and 51 kappas were shown to establish an equilibrium in mutant 51*m*3. Further, if 51*m*1 arose by mutation of 51 kappa to Sp kappa, there would be expected a strong selective advantage for the mixed kappa condition. It is possible, of course, that conditions prevailing in the stock bottle at the time the mutant arose were such as to operate against the establishment of an equilibrium between the two kinds of kappa. Finally, the fifth mutant, 47*m*1, will not be discussed in view of the fact that so little is known about the kappa from which this mutant arose. The apparent difference in interaction between the 51 and Sp kappas in 47*m*1, and the 51 and Sp kappas in 51*m*3 (as inferred from table 3) could be due

to the difference in genotype (47 and 51) with which this kappa combination is associated.

4. SUMMARY

1. The purpose of the present investigation was to determine whether changes in nuclear genes or cytoplasmic factors or both were responsible for the production of five mutations of the killer character in certain strains of *Paramecium aurelia*.

2. Four of these mutations were derived from cultures of the killer stock 51 ; the fifth arose in killer stock 47. In all cases, so far as is known, the mutations were of spontaneous origin.

3. The five mutants differed from the parent stocks in either the type of killing or in the strength of killing as measured by the numbers of sensitive animals killed in a given period of time. These differences were maintained throughout vegetative and sexual reproduction.

4. Two general types of killing action were manifested by the mutant stocks. The first type was identical with that produced by the original 51 parent. The second type differed strikingly from stock 51, causing affected animals to spin violently prior to death.

5. Animals whose killing was of one type were resistant to killing action of that type but not to killing action of the other type. Animals whose killing was of both types were completely resistant to both types.

6. A series of crosses demonstrated that : (*a*) the presence of a " killer " gene in each mutant stock was necessary for determination of mutant killing, (*b*) the presence of a kappa-type of cytoplasmic factor in each mutant stock was also necessary for determination of the killer character, (*c*) in the presence of the killer gene, *K*, from the original stock 51, the mutant killer characters were maintained, (*d*) in the presence of the killer gene from each mutant stock, the original 51 killer character was preserved, (*e*) the killer gene in each mutant stock must then be effectively identical with the *K* gene in the original stocks from which they were derived.

7. The results expected if the mutant killing had been due to mutation of the *K* gene or to some other gene influencing the killer character were not obtained. On the contrary, the results showed that the mutants did not differ from the original in any genes affecting this trait.

8. Three distinct kinds of kappa were separated out from the mutants and obtained in pure form in killer cells. This was accomplished by dilution of the kappa concentration in the mutants to a single particle per animal, followed by slow growth of the animal to permit the particle to restore the maximal kappa concentration.

9. Three explanations of the origin of the different kappas were considered : *de novo* origin, infection, and mutation of the kappa originally present. As the first two could be effectively excluded, it was concluded that the physical basis of the killer character mutations was mutation of the kappas in the original stocks.

10. One of the three types of kappa found in the mutant stocks controlled original 51-type killing, existed in relatively low concentration within the killer animal, and multiplied as rapidly as the cell itself.

A second type of kappa, Sp, controlled the spinner type of killing, was present in high concentration in the killers, and multiplied more slowly than 51 kappa.

The third kind, 51*r* kappa, controlled extremely weak 51 killing, or none at all, but it conferred resistance to 51 paramecin. This kappa was able to multiply as rapidly as 51 kappa and its concentration per cell more nearly resembled that of 51 kappa than the Sp kappa.

11. Among the five mutant stocks, 51*m*1 was pure for Sp kappa, and 51*m*5 was pure for 51*r* kappa. The remaining three mutants each contained two kinds of kappa: 51*m*3 contained 51 and Sp kappas, 51*m*2 contained 51*r* and Sp kappas, and 47*m*1 contained 51 and Sp kappas. The latter two mutants thus not only contained two kinds of kappa but both differed from the original kappa.

12. Within a cell and its progeny, under standard conditions, certain combinations of two kinds of kappa (viz. Sp and 51, or Sp and 51*r*) attain and maintain an equilibrium population. On the other hand, there are indications that 51*r* kappa possesses a selective advantage over 51 kappa, displacing the latter from a cell when these two types occur together. These relations among the kappa types provide a plausible explanation for the establishment and detection of mutants 51*m*2, 51*m*3 and 51*m*5.

5. REFERENCES

DELAMATER, E. D. 1948.
Basic fuchsin as a nuclear stain for fungi.
Mycologia, 40, 423-429.

DIPPELL, RUTH V. 1948.
Mutations of the killer plasmagene, kappa, in variety 4 of *Paramecium aurelia*.
Amer. Nat., 82, 43-50.

GOLDSTEIN, L. 1949.
Contribution a l'étude de la sensibilité héréditaire au gaz carbonique chez la Drosophile. Mise en évidence d'une forme nouvelle du génoide.
Bulletin Biologique de la France et de la Belgique, 83, 177-188.

PREER, J. R. 1946.
Some properties of a genetic cytoplasmic factor in Paramecium.
P.N.A.S., 32, 247-253.

PREER, J. R. 1948*a*.
A study of some properties of the cytoplasmic factor, "kappa," in *Paramecium aurelia*, variety 2.
Genetics, 33, 349-404.

PREER, J. R. 1948*b*.
Microscopic bodies in the cytoplasm of "killers" of *Paramecium aurelia* and evidence for the identification of these bodies with the cytoplasmic factor, kappa.
Genetics, 33, 625.

Plate 1

Explanation of figures

Figs. (*a*) to (*i*).—Photomicrographs of the morphological changes undergone by sensitive animals exposed to paramecins of three different killer stocks. Figs. (*a*), (*b*), (*f*), (*g*), (*h*) and (*i*) : living animals. Figs. (*c*), (*d*) and (*e*) : fixed and stained preparations. (The large, deeply staining body is the macronucleus.) Background has been blocked out in all figures except (*f*). Approximate magnification : ×200.

Effect of stock 51 paramecin

(*a*) and (*b*) early stages of effect, showing small blisters that appear and enlarge on body surface.

(*c*) and (*d*) development of the large posterior " hump," the most characteristic of the responses by sensitives to 51 paramecin.

(*e*) final stage of killing effect. Animals decrease in size to at least one-half of that shown in (*a*) and assume a pear-shaped to spherical form prior to death.

Effect of stock 47 paramecin

(*f*) characteristic response : development of a single large vacuole in posterior part of body. (This photograph was obtained by Dr T. M. Sonneborn prior to loss of killer character by stock 47.)

Effect of mutant stock 51m1 paramecin

(*g*) and (*h*) vacuolation of sensitives subjected to 51*m*1 paramecin.

(*i*) final stage of killing action, characterised by paralysis or formation of huge blister on body surface into which body contents may subsequently empty.

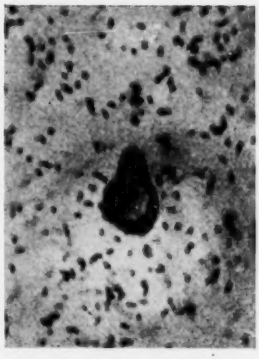
(a)

(b)

(c)

(d)

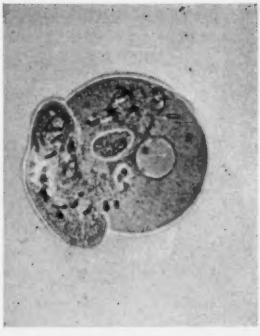
(e)



(f)

(g)

(h)



(i)

PREER, J. R. 1950.
Microscopically visible bodies in the cytoplasm of the "killer" strains of *Paramecium aurelia*.
Genetics, *35*, 344-362.

RHOADES, M. M. 1946.
Plastid mutations.
Cold Spring Harbor Symp. Quant. Biol., *11*, 202-207.

SONNEBORN, T. M. 1939.
Paramecium aurelia: mating types and groups; lethal interactions; determination and inheritance.
Amer. Nat., *73*, 390-413.

SONNEBORN, T. M. 1940.
The relation of macronuclear regeneration in *Paramecium aurelia* to macronuclear structure, amitosis and genetic determination.
Anat. Rec., *78*, 53-54.

SONNEBORN, T. M. 1943.
Gene and cytoplasm. I. The determination and inheritance of the killer character in variety 4 of *P. aurelia*. II. The bearing of the determination and inheritance of characters in *P. aurelia* on the problems of cytoplasmic inheritance, Pneumococcus transformations, mutations and development.
P.N.A.S., *29*, 329-343.

SONNEBORN, T. M. 1944.
Exchange of cytoplasm at conjugation in *Paramecium aurelia*, variety 4.
Anat. Rec., *89*, 49.

SONNEBORN, T. M. 1945.
The dependence of the physiological action of a gene on a primer and the relation of primer to gene.
Amer. Nat., *79*, 318-329.

SONNEBORN, T. M. 1946.
Experimental control of the concentration of cytoplasmic factors in Paramecium.
Cold Spring Harbor Symp. Quant. Biol., *11*, 236-255.

SONNEBORN, T. M. 1947*a*.
Recent advances in the genetics of Paramecium and Euplotes.
Advances in Genetics, *1*, 263-358.

SONNEBORN, T. M. 1947*b*.
Developmental mechanisms in Paramecium.
Growth Symposium, *11*, 291-307.

SONNEBORN, T. M. 1948.
Symposium on plasmagenes, genes and characters in *Paramecium aurelia*. Introduction.
Amer. Nat., *82*, 26-34.

SONNEBORN, T. M. 1950*a*.
Methods in the general biology and genetics of *Paramecium aurelia*.
J. Exp. Zool., *113*, 87-148.

SONNEBORN, T. M. 1950*b*.
The cytoplasm in heredity.
Heredity, *4*, 11-36.

SONNEBORN, T. M., AND DIPPELL, RUTH V. 1946.
Mating reactions and conjugation between varieties of *Paramecium aurelia* in relation to conceptions of mating type and variety.
Physiol. Zool., *19*, 1-18.

COMPARATIVE GENETICS OF *DROSOPHILA PROSALTANS*

B. SPASSKY, S. ZIMMERING and TH. DOBZHANSKY
Columbia University, New York

Received 25.xii.49

1. INTRODUCTION

THE architecture of the germ plasm undergoes changes in the process of evolution. The genes become altered by mutation, and their arrangement in the chromosomes is modified by structural change. Comparison of the genetic maps of chromosomes in related species is one of the methods of studying the evolution of the germ plasm. Species of *Drosophila* are favourable for such studies, and about ten of them have been investigated in more or less detail. It appears that the degree of divergence in systematic characters is not very strongly correlated either with the amount of gene differentiation, or with divergence in the gene arrangement. The information now available is, however, insufficient to give a clear idea about the mutual relationships of the various classes of changes that are observed. More data is obviously necessary. No species of the *saltans* group of the subgenus *Sophophora* has been studied. Since 1944, we have collected mutants and examined the linkage relationships in *Drosophila prosaltans* Duda, which is a member of this group. The results are reported in the present article.

2. MATERIAL AND METHOD

The distribution of *D. prosaltans* extends from the subtropical zone of Central Mexico to Paraguay and Southern Brazil (states of São Paulo and Paraná). Within this region, the species occurs rather sporadically; it is common only in some localities on the island of Marajó in the delta of the Amazon, and near Imperatriz, in the state of Maranhãó, Brazil. Adult flies can be collected on fallen fruit in the forest. They are attracted to fermenting banana traps, especially in advanced stages of fermentation, but they are rarely met with in human dwellings (Dobzhansky and Pavan, unpub.). In the laboratory, the species breeds well on standard food media, and is favourable material for studies on comparative genetics.

Except where otherwise specified, a strain derived from a single female collected at Belem, state of Pará, Brazil, was used in the experiments. Some mutants arose spontaneously in the course of the work, but most of them were found in the progeny of flies treated with X-rays. Young males were given 4000 *r*-units of X-rays, and

outcrossed to untreated females, using 3-5 pairs of flies per culture. The F_1 flies were examined for dominant mutants. Then two or three small mass cultures, with about 4 pairs of flies, were made from each F_1 bottle, and the F_2 and F_3 flies were examined for sex-linked and autosomal-recessive mutants. All the experiments were carried out at room temperature or in incubators at 25° C. The X-ray treatments were administered by Drs L. D. Marinelli and E. Focht of the Memorial Hospital in New York, to whom the authors wish to express their gratitude.

3. SEX-LINKED MUTANTS

cut (*ct*).—In an X-rayed culture, 29th April 1947. Recessive. All wing margins cut, making the wing narrow and acuminate at the distal end, lancet-like in shape. This shape resembles the wing of the autosomal dominant mutant Blade of *D. pseudoobscura*. Among the cut alleles in *D. melanogaster*, it resembles most cut [6] (fig. 14 in Morgan, Bridges and Sturtevant, 1925) but is more acuminate.

Eyeless (*Ey*).—In an X-rayed Guatemala strain, 16th April 1944. Dominant, lethal in the male. Eyes variable in size from normal to no eye at all. Asymmetrical expression very frequent, including extreme cases when a normal eye is present on one side and none on the other. The eye surface normal, not rough when eye is large, but the eye may be misshapen when its size is much reduced. Antennæ deformed or reduplicated in some flies. In some completely eyeless individuals, the front and the vertex are also reduced and warped. This mutant seems to be a good parallel to the dominant mutant Eyeless in the fourth chromosome of *D. melanogaster*.

facet (*fa*).—13th March 1947, in an X-rayed culture. Recessive. Eyes uniformly rough, the roughness being more pronounced in males than in homozygous females. Analogy with facet in *D. melanogaster* uncertain. An allelic mutant was found on 15th March 1944, in the offspring of an X-rayed father; this proved to be connected with a translocation between the X and the second chromosomes.

garnet (*g*).—13th April 1947, in an X-rayed culture. Recessive. Eye colour in young flies between vinaceous-rufous and Hay's russet (Ridgway, plate XIV), darkens slightly with age. Testes very pale yellow. Malpighian tubes almost completely clear, larval Malpighian tubes clear. The compound garnet-sepia differs little from garnet. May or may not be analogous to garnet in *D. melanogaster*.

grooveless (*gvl*).—27th April 1947, in an X-rayed culture. Recessive. The depression normally present along the suture dividing the scutellum from the thorax almost completely obliterated. The flies usually have black pigmented scars on the sides of the scutellar suture or above the humeral callus, or on the pleuræ behind the sternopleural bristles. The scars are, as a rule, asymmetrical. In the young flies, they contain a liquid which is probably blood. These scars arise from blisters that are formed in certain parts of the integument which are filled with blood during the hatching from the pupa. An excellent parallel to grooveless in the fourth chromosome of *D. melanogaster*.

miniature (*m*).—27th April 1947, in an X-rayed culture. Recessive.

Wings decreased in size but the proportions are only slightly different from normal (slightly narrower). The density of wing hairs increased, which indicates a reduction in cell size. An excellent parallel to miniature in *D. melanogaster*.

Notch (*N*).—February 1944, in an X-rayed culture. Dominant, lethal in the male. Wing veins thickened and expanded into small deltas at the wing margins. A strong notch on the first section of the costa making the wing narrow at the base, and sometimes small notches between the ends of the third and fourth veins. Acrostichal bristles normal, but the inner longitudinal dark bands on the thorax constricted or even interrupted at the level of the central spot. This mutant differs considerably in appearance from Notch in *D. melanogaster*, and yet it has enough common features to be regarded as a good analogue. Recurrences of Notch observed on 27th April 1947 and 1st April 1948, in X-rayed strains.

Prune (*Pn*).—February 1948, in an X-rayed culture. Dominant, lethal in the male. Eyes in young flies between Ridgway's garnet-brown (plate I) and Madder brown (plate XIII). The colour darkens with age to dark sepia. This mutant has no good analogues among mutants in other species.

sable (*s*).—17th April 1947, in an X-rayed culture. Recessive. Body colour much darker than normal, shiny blackish brown. The peculiar polished appearance, resembling the effect obtained when the fly is covered with oil, is particularly noticeable on the pleuræ of the thorax. The colour pattern of the thorax, which is characteristic of the species, is reduced to indistinct dark stripes. A doubtful analogue of sable in *D. melanogaster*.

scalloped (*sd*).—14th January 1947, in an X-rayed culture. Recessive. The anterior, distal, and posterior wing margins nicked to varying extents in different individuals. More extreme in males than in females. The wing veins in some flies thickened and darkened in the proximal two-thirds of the wing. Resembles beaded of *D. pseudoobscura* as well as scalloped of *D. melanogaster*.

scute (*sc*).—5th May 1948, in an X-rayed culture. Recessive. All four scutellar bristles absent, other bristles normal. Connected with a chromosomal aberration which suppresses recombination in the X chromosome. Probably analogous to scute in *D. melanogaster*.

sepia (*se*).—20th April 1947, in an X-rayed culture. Recessive. Eye colour purplish-sepia, translucent in young flies, darkens with age. Colour of testes in adults normal. Colour of Malpighian tubes in adults and larvæ normal. A good analogue of sepia in *D. melanogaster*. An allelic mutant found on 29th April 1947, also from an X-rayed culture.

Shortened (*Sh*).—15th January 1947, in an X-rayed culture. Dominant, lethal in the male. The second longitudinal vein reduced distally or even wholly absent. In some flies, a section of the vein unconnected either proximally or distally is present in place of the proximal third of the normal second vein. Other veins normal. No analogue among sex-linked mutants in *D. melanogaster*.

singed (*sn*).—20th March 1944, in an X-rayed strain from Iporanga, Brazil. Recessive; homozygous female sterile. All bristles short and strongly singed or curled. Acrostichal and other hairs short, stubby, and bent or curved. Body colour slightly darker and colour pattern of thorax less distinct than in normal flies. A good parallel of the most extreme known alleles of singed in *D. melanogaster*. A recurrence of singed observed

on 18th July 1944, in the Belem strain, but this mutant possessed poor viability and was lost.

vesiculated (*vs*).—29th April 1947, in an X-rayed culture. Recessive. Wings blistered, warped or crumpled, frequently asymmetrical. The wings vary from spoon-shaped but normal in size to crumpled small rudiments. Young flies usually have large blisters filled with blood. A fair analogue of vesiculated in *D. melanogaster*.

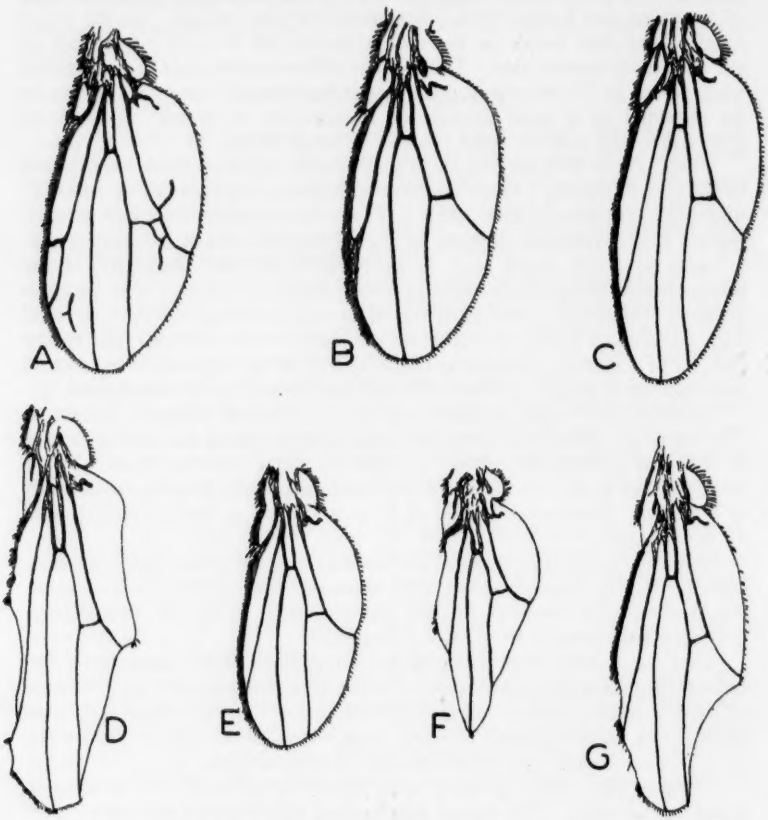


FIG. 1.—Some of the wing mutants in *Drosophila prosaltans*. A—plexus; B—Delta; C—Blade; D—Beaded; E—miniature; F—miniature-cut; G—scalloped. The magnification is similar in all cases.

white (*w*).—13th July 1944, in an X-rayed culture. Recessive. Eyes clear white. Testes and Malpighian tubes in adults and larvæ colourless. An excellent analogue of white in *D. melanogaster*.

yellow (*y*).—28th July 1944, found by Mr G. Streisinger. Spontaneous. Body colour dirty greyish yellow, almost Ridgway's clay colour or tawny olive (plate XXIX). The colour pattern on the thorax characteristic for the species is indistinct. An excellent analogue of yellow in *D. melanogaster*.

4. MUTANTS OF THE SECOND LINKAGE GROUP

Beaded (*Bd*).—17th August 1947, in the Eyeless strain. Spontaneous. Dominant, lethal when homozygous. Wings narrow and pointed at the tips ; the reduction in width is due to numerous excisions both at the costal and posterior margins. Tufts of the bristles on the costal margin preserved in some individuals, the costa is almost gone in others. The third and fourth veins preserved. A fair parallel of Beaded in *D. melanogaster*.

Curly (*Cy*).—16th June 1944, in an X-rayed culture of a strain from Bertioga, Brazil. Dominant, lethal when homozygous. Wings curved upward and outward. Manifestation variable, some flies being close to normal except for a wavyness of the wing blade, especially in the distal half. When strongly pronounced, the wing is curled 360°. Except when in compounds with plexus, no blisters are found (as in *D. pseudoobscura*) and no segmentation apparent in the wing veins (as in Curly of *D. melanogaster*) ; otherwise an excellent parallel to the similarly named mutants in those species.

Engrailed (*En*).—7th January 1948, in an X-rayed culture. Dominant, lethal when homozygous. Wings shortened, constricted at the base, and broadly rounded at the apex. The scutellum with a median nick or cleft on the posterior margin ; the posterior scutellar bristles usually shortened and frequently bent hook-shaped. This mutant has traits in common with the recessive engrailed of *D. melanogaster* and also with some alleles of vestigial in the same species.

grooved (*grv*).—6th March 1947, in a culture of Plum of Guatemala origin. Probably a spontaneous mutant. Thorax shortened and broadened, with grooves or nicks on the anterior margin, situated at the anterior ends of the inner parts of the dark stripes normally present in this species on the thorax. Scutellum short and broad, slightly bulging. Abdomen very slightly broadened. Seems not to resemble known mutants in other species.

Groundoff (*Gf*).—July 1944, in an X-rayed culture of Guatemala origin. Posterior part of the eye with normal facet structure destroyed, as though ground off with a file ; the altered part of the eye covered with a glassy cuticle. Expression more extreme at high temperature, almost suppressed at 18°. The flies with least expression have normal eyes with only a few facets or groups of facets missing near the posterior eye margin. Progressive increase of the manifestation results in a greater and greater portion of the posterior part of the eye affected, up to the posterior two-thirds in heterozygotes. The boundary between the affected and unaffected parts of the eye usually very sharp. Homozygotes usually die but some of them hatch as weak flies with the entire eye affected as described above. No parallel in other species.

Lobe (*L*).—3rd March 1947, in an X-rayed culture. Dominant, lethal when homozygous. Eyes reduced in size and strongly roughened. The reduction variable and often asymmetrical. The extreme manifestation mimics the homozygous Bar eye in *D. melanogaster* to a remarkable extent. Less extreme variants resemble more the Lobe mutant in the species just named, since they have kidney-shaped or triangular eyes which are often deformed or lobed. A side effect sometimes present is the branching of the wing veins.

plexus (*px*).—25th March 1947, in an X-rayed culture. Recessive. Wing veins branched or isolated sections of the veins present in the cells

where normally none are found. Particularly frequent are : a crossvein between the second longitudinal and the costal veins, not far from the end of the former ; an isolated vein section in the distal part of the submarginal cell ; a branching of the posterior crossvein which may develop into a vein which divides the second posterior cell into two parts ; and a recurrent vein in the third posterior cell which almost joins the anal vein. The mutant Star acts as a semi-suppressor of plexus. A fair parallel of plexus in *D. melanogaster* and *D. pseudoobscura*.

Plum (*Pm*).—June 1944, in an X-rayed Guatemala strain, and in the same month, a recurrence was observed in an X-rayed Belem strain. Dominant, lethal when homozygous. In young flies, the eye brick-red (Ridgway, plate XIII) in colour, with traces of mosaicism ; darkening with age leads to eyes of about the Hessian brown colour (Ridgway, plate XIII) and to the mosaicism being no longer noticeable. Testes yellow with transparent mosaic spots, Malpighian tubes apparently uniformly pigmented. The Plum mutant in the Belem strain is associated with a powerful crossing over suppressor, the Guatemala one seems to be free of suppression. A good parallel to Plum in *D. melanogaster*.

Star (*S*).—27th March 1947, in an X-rayed strain. Dominant, lethal when homozygous. Eyes large, strongly roughened. Exerts a suppressor effect on the mutant plexus (see above). A fair parallel to Star in *D. melanogaster*.

5. MUTANTS OF THE THIRD LINKAGE GROUP

abdomen rotatum (*ar*).—August 1947, in an X-rayed strain. Recessive. Abdomen twisted clockwise through about 45°. Males sterile, females fertile. Viability good. A good parallel to abdomen rotatum in *D. melanogaster*.

Blade (*Bld*).—February 1944, in an X-rayed strain of Bertioga (Brazil) origin. Dominant, lethal when homozygous. Wings held out at right angles to the thorax ; the shape of the wing is normal or slightly narrower than normal, sometimes unexpanded but frequently folded lengthwise or in general irregular. Resembles Dichæte in *D. melanogaster* but bristles are normal.

crossveinless (*cv*).—March 1947, in cultures which had not been recently X-rayed. Recessive. Both anterior and posterior crossveins missing or interrupted. Variable, but apparently does not overlap wild-type, since at least one of the crossveins shows interruption in all specimens. Frequently asymmetrical. Not classifiable in compound with the Delta mutant. A good parallel to crossveinless-C in *D. melanogaster*.

Delta (Δ).—A frequently recurrent mutant in X-rayed cultures. Dominant, lethal when homozygous. Wing veins thickened at the margin. Crossveins sometimes thickened. Eyes slightly rough. In weaker alleles, sometimes only the second longitudinal vein expanded in the distal portion and fused with the costal margin, so that the costal index is very radically altered. A good parallel of Delta in *D. melanogaster*.

hunchback (*hu*).—14th March 1947, in an X-rayed culture. Recessive. Thorax and scutellum short and broad, strongly convex. The groove along the suture separating the scutellum from the thorax deep. Wings curved upward or wrinkled. Variable, but does not overlap wild-type. The thorax characters are more constant than those of the wings.

Spread (*Spd*).—11th August 1947, in an X-rayed culture. Dominant, lethal when homozygous. Wings held out at right angles from the thorax, somewhat shorter and broader than normal ; frequently warped downwards.

TABLE 1

Summary of linkage data. Three point crosses

Nature of the cross	0	1	2	1, 2	Total
y 1 2 *w* / 1 *sn* 2	100-145	97-35	87-38	18-22	542
y 1 2 *w* / 1 *ct* 2	220-289	37-20	162-111	3-10	852
y 1 2 *w* / 1 *fa* 2	205-240	56-46	142-52	2-9	752
gvl 1 2 / 1 *y* 2 *w*	196-123	5-35	46-231	9-7	652
y 1 2 *w* / 1 *vs* 2	62-131	57-31	25-7	4-3	320
ct 1 *m* 2 / 1 2 *vs*	424-220	19-54	15-30	1-0	763
se 1 *y* 2 *m* / 1 *sc* 2	137-116	42-49	13-10	4-10	381
y 1 2 *w* / 1 *s* 2	104-82	15-52	52-2	1-5	313
y 1 2 *w* / 1 *sd* 2	221-214	89-81	1-1	0-0	607
se 1 2 / 1 *ct* 2 *sn*	96-79	58-77	12-16	9-8	355
m 1 2 *vs* / 1 *s* 2	271-38	52-7	2-6	1-0	377
y 1 2 *vs* / 1 *m* 2 / 1 *cv* 2	88-101	14-17	25-46	3-1	295
△ 1 2 *Spd* / 1 *hu* 2	408-382	54-4	75-80	8-0	1011
△ 1 2 *Spd*	51-134	46-10	5-1	1-1	249
△ 1 *Spd* 2 / 1 2 *ar*	308-126	82-224	40-78	71-23	952

Aristapedia (*Arp*).—8th November 1947, in an X-rayed culture. Dominant, viability poor even in heterozygous condition, lost. An extreme aristapedia-like change with aristæ transformed into a leg-like organ.

6. LINKAGE MAPS

Drosophila prosaltans has three pairs of chromosomes ; the X, Y, and second chromosomes are large and metacentric, the third chromosome is slightly more than half as long as the others and acrocentric. In accordance with this, three linkage groups have been found. The largest of them consists of 17 sex-linked genes, which must be carried in the X-chromosome. The second linkage group contains 9 genes and the third contains 7 genes. Prof. A. G. L. Cavalcanti (unpub.) has examined the chromosomes in the salivary

gland cells of some of our mutants which were known to be associated with structural changes and proved that the second linkage group is borne in the metacentric, and the third linkage group is borne in the acrocentric autosomes.

TABLE 2

Summary of linkage data. Four point crosses

Nature of the cross	0	1	2	3	1, 2	1, 3	2, 3	1, 2, 3	Total
y 1 2 3 *w* 1 *ct* 2 *sn* 3	693-654	242-209	132-119	10-11	6-4	1-5	10-5	1-2	2104
1 *ct* 2 3 *sn*	282-301	88-92	49-44	1-3	2-1	0-0	0-0	0-0	863
y 1 2 *m* 3 1 *fa* 2 3 *w*	77-126	32-11	36-17	26-20	4-3	1-6	2-1	0-0	362
y 1 2 *vs* 3 *se* 1 *y* 2 *m* 3	120-57	10-33	10-12	2-28	16-23	21-23	0-3	0-2	360
1 2 3 *g*	162-109	63-125	41-17	45-47	12-25	23-34	13-11	0-5	732
se 1 2 *y* 3 *m* 1 *gvl* 2 3	327-401	137-86	46-104	13-43	2-1	4-6	5-4	0-0	1179
1 2 *g* 3	230-385	90-63	104-81	?-93	2-3	?-12	0-3	0-0	1066
y 1 *m* 2 3 *vs* *y* 1 *m* 2 3 *w*	95-25	12-0	42-5	43-7	6-8	1-0	0-0	0-0	244
1 2 3 *Pn*	479-748	299-196	12-3	203-179	0-7	6-31	4-15	1-4	2187
gvl 1 *y* 2 *m* 3 *S* 1 2 *Bd* 3 *Cy*	185-166	0-11	12-28	52-46	4-3	0-3	0-8	1-1	520
1 *px* 2 3 *Gf* 1 *Pm* 2 3 1 2 *S* 3 *Bd*									

TABLE 3

Summary of linkage data. Five point crosses

Interval	*se* 1 *gvl* 2 *y* 3 4 *m* 1 2 3 *N* 4	*Gf* 1 *Pm* 2 3 *grv* 4 1 2 *S* 3 4 *Cy*
0	17-46	720-665
1	33-14	0-0
2	5-8	108-201
3	0-3	3-7
4	2-6	47-171
1, 2	1-1	0-0
1, 3	2-0	0-0
1, 4	4-1	1-0
2, 3	0-0	3-2
2, 4	0-1	54-21
3, 4	0-0	6-1
2, 3, 4	0-0	2-4
Total	144	2016

Tables 1-3 report the results of linkage experiments in which at least three genes of the same linkage group were involved. The numbers of the flies in the two complementary classes are given for

each interval ; a convention is followed of giving first the class which contains the gene indicated on the left above the line in the formula describing the nature of the cross. The data are summarised in the form of linkage maps in fig. 2.

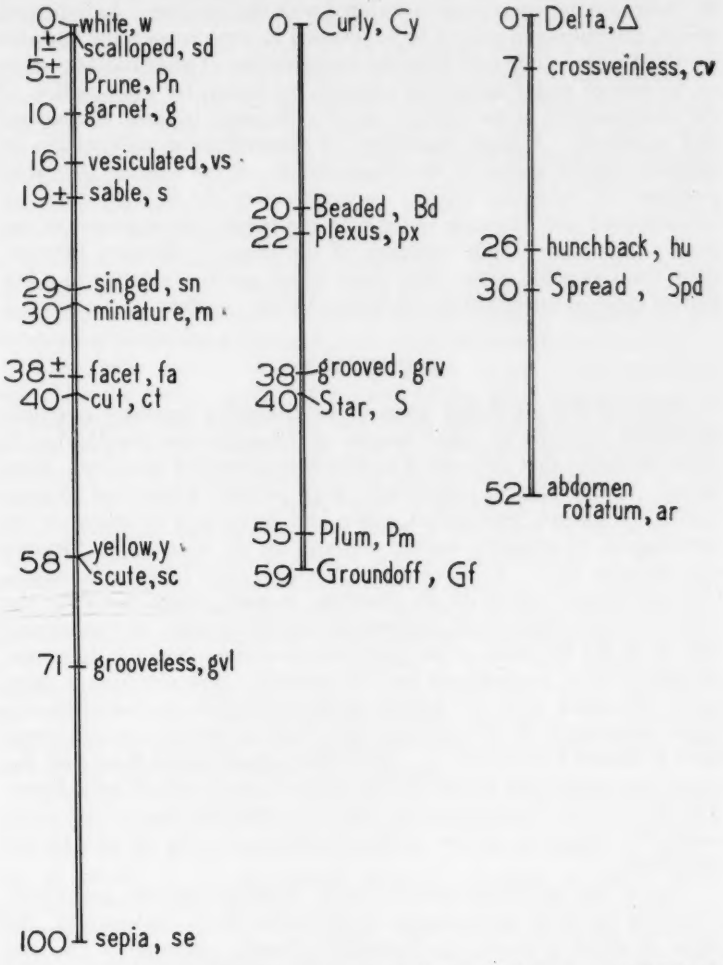


FIG. 2.—Linkage maps of the X-chromosome (left), second chromosome (middle), and third chromosome (right) of *Drosophila prosaltans*.

Examination of the data discloses a rather startling variability of the recombination percentages between the same genes in different experiments. Thus, the frequencies of recombination between yellow

(*y*) and miniature (*m*) varied from 9·7 to 24·3 per cent.; between singed (*sn*) and white (*w*) from 2·1 to 30·4 per cent.; and between Star (*S*) and Curly (*Cy*) from 16·7 to 41·6 per cent. Since most of the mutants were induced by X-ray treatments, a suspicion arose that the variability of the recombination percentages might be caused by chromosomal aberrations associated with the mutants. As indicated above, this suspicion proved to be justified in some cases; the mutants involved were eliminated from the experiments. Other mutants seem to be free of major structural changes, as shown by examination of the chromosomes in the salivary gland cells made by Prof. Cavalcanti and ourselves. A high variability of recombination percentages in different strains seems to be characteristic of the species *Drosophila prosaltans* (*cf.* Spurway, 1945). In constructing the linkage maps, we concentrated our attention on finding the proper arrangement of the genes, and made rough estimates of the relative distances between them from variable data. The genes whose position relative to other known genes is uncertain are indicated by the ± signs.

7. DISCUSSION

Some of the sex-linked mutants in *Drosophila prosaltans* resemble sex-linked mutants in other species of *Drosophila* so closely that it seems probable that changes in homologous genes are involved. Here belong white, singed, miniature, cut, yellow, scute and Notch. Scalloped, garnet, vesiculated, sable and facet may or may not be homologous to similarly named mutants in *D. melanogaster* (Bridges and Brehme, 1944; Sturtevant and Novitski, 1941; Spurway, 1945). One sex-linked mutant in *D. prosaltans*, namely, sepia, parallels the sepia mutants which in *D. melanogaster* and *D. simulans* are autosomal and lie in the left limb of the third chromosome. Sepia is, however, sex-linked in *D. pseudoobscura* and *D. persimilis*. The situation is quite easily explained, since the species in which sepia is sex-linked have a large metacentric X-chromosome and those in which it is autosomal have a shorter acrocentric X; the former species have three and the latter four autosomal strands in the salivary gland cells of male larvæ. The metacentric X-chromosome contains probably most of the genic materials which in species with acrocentric X's lie in one of the autosomes.

One of the sex-linked mutants in *D. prosaltans*, namely, grooveless, is almost certainly homologous to grooveless in *D. melanogaster*, the locus of which is in the small dot-like (fourth) autosome. The sex-linked Eyeless of *D. prosaltans* also resembles the Eyeless in the dot-like autosome of *D. melanogaster*. This situation is not entirely unexpected, since the species of the subgenus *Sophophora* which are native in the Neotropical zoogeographic region (*i.e.* the species of *saltans* and *willistoni* groups) lack the dot-like autosome which is present in most species of other sections of the genus. Sturtevant and Novitski (1941)

conjectured that the genic materials of the dot-like autosome form in *D. willistoni* form a short " arm " attached to the same centromere as the approximately acrocentric third chromosome. This conjecture is not supported by the facts known in *D. prosaltans*, in which at least a part of the dot-like autosome is incorporated into the X-chromosome (see, however, the discussion of the genes in the third chromosome, below).

The second linkage group in *D. prosaltans* contains the mutants Curly, plexus, Star and Plum which are similar enough to the identically named mutants in the second chromosome of *D. melanogaster* to make probable that homologous loci are involved. Engrailed and Lobe are two further mutants which resemble second-chromosome mutants in *D. melanogaster*. The second chromosomes of the two species carry mostly similar materials. The mutant Beaded in the second chromosome of *D. prosaltans* resembles, however, Beaded in the right limb of the third chromosome of *D. melanogaster*. The resemblance is not close enough to insure homology, and the hypothesis that the second chromosome of *D. prosaltans* carries some materials located in *D. melanogaster* in the third chromosome cannot be accepted as proven.

The Delta mutant in the third linkage group of *D. prosaltans* resembles the Deltas in the right limbs of the third chromosomes of *D. melanogaster* and *D. simulans*, and Delta and Smoky in the second chromosomes of *D. pseudoobscura* and *D. persimilis*, all of which are presumed to be homologous. Crossveinless and Aristapedia of *D. prosaltans* are likely to be homologous respectively to crossveinless-*b* and aristapedia, also in the right limb of the third chromosome of *D. melanogaster*, and hunchback is possibly homologous to the not precisely located third-chromosome mutant humped in *D. melanogaster* (Bridges and Brehme, 1944, p. 93). Spread of *D. prosaltans* resembles spread which is supposed to lie in the left limb of the third chromosome of *D. melanogaster* (Bridges and Brehme, p. 178), and Blade is not unlike Dichaete in the same limb. The possibility must, therefore, be reckoned with that the third linkage group of *D. prosaltans* contains genes some of which lie in the right and others in the left limb of the third chromosome of *D. melanogaster*.

Most interesting is the abdomen rotatum mutant in the third linkage group of *D. prosaltans*. It is quite similar to abdomen rotatum mutants which are found in the dot-like autosomes of *D. melanogaster*, *D. affinis*, and *D. virilis*. In *D. melanogaster* abdomen rotatum is linked with grooveless and with Eyeless, which are in the X-chromosome of *D. prosaltans* (see above). The genic materials borne in the dot-like autosomes in *D. melanogaster* are, in *D. prosaltans*, divided between the X and the third chromosomes. The notion of indivisibility of the chromosome " elements " in the phylogeny of *Drosophila* is invalid (Sturtevant and Novitski, 1941).

The arrangement of the genes within the chromosomes of *D. prosaltans* will be discussed in the companion article of Spassky and Dobzhansky which deals chiefly with *D. willistoni*.

8. SUMMARY

Drosophila prosaltans has three groups of linked genes which correspond to the three pairs of chromosomes present in its cells. These linkage groups have been compared to those of *D. melanogaster*. The sex-linked genes of *D. prosaltans* are of three kinds. Most of them correspond to genes borne in the X-chromosome of *D. melanogaster*; sepia is probably a homologue of the sepia mutant in the left limb of the third chromosome of *D. melanogaster*; and, finally, grooveless and Eyeless are borne in the dot-like autosome (fourth chromosome) of the latter species. The second chromosome of *D. prosaltans* corresponds to the second, and the third to the right limb of the third chromosome of *D. melanogaster*. However, the gene abdomen rotatum in the third linkage group of *D. prosaltans* is probably a homologue of the similarly named mutant in the fourth chromosome of *D. melanogaster*. Junction, fragmentation, and it seems also breakup and redistribution of gene aggregates, have taken place in the phylogeny of *Drosophila*.

9. REFERENCES

BRIDGES, C. B., AND BREHME, K. S. 1944.
The mutants of *Drosophila melanogaster*.
Carnegie Inst. Washington, Publ. 552, 1-257.

MORGAN, T. H., BRIDGES, C. B., AND STURTEVANT, A. H. 1925.
The genetics of Drosophila.
Bibliogr. Genetica, *2*, 1-262.

RIDGWAY, R. 1912.
Color standards and color nomenclature. Washington.

SPURWAY, H. 1945.
The genetics and cytology of *Drosophila subobscura*. I. Element A.
J. Genet., *46*, 268-286.

STURTEVANT, A. H., AND NOVITSKI, E. 1941.
The homologies of the chromosome elements in the genus Drosophila.
Genetics, *26*, 517-541.

COMPARATIVE GENETICS OF *DROSOPHILA WILLISTONI*

B. SPASSKY and TH. DOBZHANSKY
Columbia University, New York

Received 26.xii.49

1. INTRODUCTION

Drosophila willistoni is one of the species which were used in the pioneer studies on comparative genetics of *Drosophila*. Lancefield and Metz (1922) and Ferry, Lancefield and Metz (1923) reported a number of mutants, published a linkage map of the X-chromosome, and suggested that only about half of the X of this species corresponds to the X-chromosome of *D. melanogaster*, while the other half of the X of *D. willistoni* carries genes which are autosomal in *D. melanogaster*. The work on *D. willistoni* was then abandoned. Sturtevant and Novitski (1941) re-analysed the published information and concluded that one of the two limbs of the X-chromosome of *D. willistoni* corresponds to the X, and the other limb to the left part of the third chromosome of *D. melanogaster*. The homologies of the other chromosomes remained obscure.

The mutant strains of Metz and his collaborators are no longer in existence. Because *D. willistoni* is very favourable for investigations on the population genetics of tropical species, we initiated in 1944 a new collection of mutants and the construction of linkage maps. The results are reported in the present article.

2. MATERIAL AND METHOD

D. willistoni is one of the commonest species of *Drosophila* in the American tropics, and it is found from the southern tip of Florida to Southern Brazil (the state of Rio Grande do Sul). Strains from the same, and especially from different, geographic regions very often differ by inversions of blocks of genes in their chromosomes. The geographic origin of the material with which the genetic work is done is therefore important. Most of our mutants were obtained in a strain from Belem, Pará, Brazil, which was known to be free of inversions : that is to say it is homogeneous in respect of inversions. Metz and his collaborators worked with a strain from Cuba, which almost certainly differed from ours in gene arrangement. Some of our mutants induced by X-ray treatment proved to be associated with chromosomal aberrations ; the same is true for a few mutants which were found in strains of other than Belem origin. These mutants are unfit for construction of linkage maps, except when they are

freed from association with the chromosomal aberrations by crossing over with the standard Belem chromosomes. Except where otherwise specified in the descriptions of the mutants, the latter are of Belem origin and free of major chromosomal aberrations. The technique of the work with *D. willistoni* has been the same as described in the article by Spassky, Zimmering and Dobzhansky, dealing with *D. prosaltans* and published in the same issue of this journal.

3. SEX-LINKED MUTANTS

copper (*cp*).—March 1945, in an X-rayed culture. Eye colour Brazil red (Ridgway, plate I), age change slight. Testis sheath pale yellow. Posterior scutellar bristles form an angle of about 45° with the plane of the scutellum. Homozygous females sterile. This combination of traits is not, as far as we know, found in any mutant in other species of *Drosophila*.

cut (*ct*).—September 1946, spontaneous. Wing margins notched (fig. 1). Resembles cut [13] in *D. melanogaster* (fig. 14, Morgan *et al.*, 1925), and probably indicates homology of the loci. May or may not be allelic to beaded of Lancefield and Metz (1922).

echinus (*ec*).—May 1945, spontaneous. Eyes very rough, facets rounded and very convex, but the rows of the facets fairly regular. Eyes large, slightly bulging. Body build and bristles normal. Possibly this is an allele of rough of Lancefield and Metz (1922). Among the mutants of *D. melanogaster*, resembles most the second-chromosome recessive, echinoid, rather than the sex-linked echinus.

eosin (w^e).—June 1944, in an X-rayed culture. Eye colour between grenadine red (Ridgway, plate II) in young, and pompeian red (plate XIII) in old flies. Testes colourless. An allele of white. No sexual dimorphism. The eosin-prune compound has eyes about capucine orange (plate III). The orange mutant of Lancefield and Metz (1922) was in all probability also an allele of white.

forked (*f*).—June 1945, in an X-rayed culture. Bristles shortened and irregularly bent, some of them, particularly the posterior dorsocentrals and the scutellars, forked or singed at the tip in most flies. Resembles some weak alleles of forked in *D. melanogaster*, and may be homologous. The difficulty of establishing the homology is increased by the existence in our material of a phenotypically very similar, but not allelic, mutant called singed (see below). Sturtevant and Novitski (1941) believed that the stubby mutant of Lancefield and Metz (1922) is a homologue of forked of *D. melanogaster*, but, judging from the description, stubby is rather different from our forked.

Incomplete (*Inc*).—September 1944, in an X-rayed culture. The fourth and fifth wing veins partly or wholly missing between the crossvein and the margin (fig. 1). Viable and more extreme in homozygous than in heterozygous condition, and highly variable in both. The weakest expression differs from wild type only in having the posterior crossvein oblique instead of at right angles to the veins. The strongest expression destroys the crossvein as well as the longitudinal veins distal to it, and a part of the fourth vein proximal to the crossvein. No analogues in other species.

lozenge (*lz*).—February 1945, in an X-rayed culture. Eyes smaller than normal, facets rough and fused, the eye surface as though varnished, the

eye margin lighter than the centre, in some individuals dark specks scattered over the eye. Although less extreme than most alleles of lozenge in *D. melanogaster*, this mutant is probably homologous. Judging from the description, resembles also morula of Lancefield and Metz (1922), but the locus of morula in the chromosome indicated by these authors is quite different from ours (see below).

miniature (*m*).—March 1945, in an X-rayed culture. Wings slightly more than half as long as normal (fig. 1), of normal proportions, opaque because of the high density of the wing hairs. Females sterile. An excellent parallel of miniature in *D. melanogaster*.

Notch (*N*).—April 1945, in an X-rayed culture. The third to the fifth longitudinal veins considerably thickened. Thoracic hair disarranged. No notching of the wing margin. Lethal in the male and associated with an inversion which suppresses crossing over from Incomplete to roughoid (fig. 2). Despite the unusual phenotype (absence of notching of the wing margin), probably homologous to Notch in *D. melanogaster* and several other species.

prune (*pn*).—May 1944, in an X-rayed culture. Eye colour coral red (Ridgway, plate XIII) in young flies, rapidly darkening with age to about Morocco red (plate I). Testes yellow but lighter than in normal males. Since this mutant phenotype resembles several sex-linked and autosomal mutants in *D. melanogaster*, the homology is an open question.

reduced veins (*rdv*).—April 1944, in an X-rayed culture of Rio de Janeiro origin. All wing veins strongly reduced (fig. 1), except that the costa and a part of the third longitudinal vein are always preserved. Viability fair in the male, but lethal in homozygous females. This is perhaps the most extreme known case of a sexual difference in the viability effect of a mutant in *Drosophila*. Probably this is an extreme allele of the mutant called short by Lancefield and Metz (1922), and possibly a homologue of veinlet in *D. melanogaster*.

rose (*rs*).—November 1946, spontaneous. Eyes cornelian red to rufous (Ridgway, plate XIV). In the eosin-rose compound, the eye colour light ivory. The mutant was lost before the colour of the testis sheath had been studied. Homology ambiguous ; among the mutants in the left limb of the third chromosome of *D. melanogaster*, remotely resembles rose, among the sex-linked mutants, not unlike prune, carnation, or ruby.

roughoid (*ru*).—March 1945, in an X-rayed culture. Eyes rough, normal in size and shape, no black erupted facets. A possible parallel to roughoid in *D. melanogaster*.

scute (*sc*).—August 1945, in an X-rayed culture. The anterior scutellars, and one or both posterior scutellar bristles missing. A good parallel to scute of *D. melanogaster*. Like scute of Lancefield and Metz (1922).

sepia (*se*).—April 1945, in an X-rayed culture. Eye colour somewhat more brownish and more translucent than normal in young flies, darkening with age to Hay's maroon (Ridgway, plate XIII). Testis sheath bright yellow. Some of the scutellar bristles are missing in homozygous females. Although the eye colour in old flies is lighter than in sepia of *D. melanogaster*, these mutants are probably homologous.

short (*sh*).—April 1944, in an X-rayed culture. The fifth vein does not reach the margin. Scutellum with a small but constant nick on the posterior margin between the scutellar bristles. The venation is variable ; in some flies the fifth vein is complete but the end section thinner than

the rest of the vein. Posterior crossvein often arched. Normally recessive but behaves as a dominant in females heterozygous for short and for reduced. No clear parallel in other species; the identically named mutant in *D. pseudoobscura* probably not homologous.

singed (*sn*).—April 1945, in an X-rayed culture. Phenotypically indistinguishable from forked (see above), but not allelic. Homozygous female fertile. Probably homologous to either singed or to forked of *D. melanogaster*, and to forked found by Lancefield and Metz (1922) in *D. willistoni*.

stubbloid (*sb*).—April 1944, in an X-rayed culture of Rio de Janeiro origin. Bristles about half as long, but as thick or even thicker than normal, tapering at the ends. Wings normal. Homozygous females sterile. Phenotypically resembles very closely stubbloid in the right limb of the third chromosome of *D. melanogaster*; this resemblance is closer than that to javelin in the left limb of the third, and much closer than to kurz or small bristle in the X-chromosome of *D. melanogaster*, although all these mutants reduce the length of the bristles. Clearly differs from stubby of Lancefield and Metz (1922).

veinlet (*ve*).—July 1947, spontaneous. The second, third, and fourth veins, especially the latter two, abbreviated. The fifth vein usually not affected. Almost certainly identical with abbreviated of Ferry, Lancefield and Metz (1923), and doubtfully homologous to veinlet in *D. melanogaster*.

white (*w*).—January 1945, in an X-rayed culture. Eyes white, testes transparent. Viability poor. An allele of eosin. An excellent homologue of white in *D. melanogaster*.

yellow (*y*).—March 1947, in an X-ray culture. Body, bristles, and wings yellow. An excellent parallel to yellow of *D. melanogaster*.

4. MUTANTS OF THE SECOND LINKAGE GROUP

abbreviated (*abb*).—March 1944, spontaneous. Some of the macrochaetæ absent, but the basal rings are preserved. Highly variable but does not overlap wild type; orbitals, postverticals, ocellars, dorsocentral or scutellar bristles may be absent; head bristles absent more often than those on the thorax. Asymmetry very frequent. Among the mutants of *D. melanogaster* resembles most rasé in the right limb of the third chromosome, much less similar to abbreviated in the second chromosome.

brown (*bw*).—July 1944, in an X-rayed culture of Rio de Janeiro origin. Eye colour vinaceous-rufous (Ridgway, plate XIV), slightly darker in old flies. Wings usually wavy, but normal in some flies. Testis sheath transparent. The eye colour resembles that in brown of *D. melanogaster*.

Emarginate (*Em*).—February 1945, in an X-rayed culture of Belem origin. Eyes decreased to about half of normal size, often with a notch on the anterior margin, the facets roughened and disarranged in the vicinity of the notch. Lethal when homozygous. Resembles Emarginate in *D. pseudoobscura* (third chromosome); homology with Lobe of *D. melanogaster* (*cf.* Sturtevant and Tan, 1937) doubtful.

Hook (*Hk*).—August 1944, in an X-rayed culture of Bertioga origin. Some of the bristles on the thorax or on the scutellum hooked or bent at right angles, most often near the tip. Some bristles remain straight but suddenly diminished in diameter before reaching the tip. Dorsocentrals

sometimes bent crescent-like. Viable in homozygotes. Very variable but seldom overlapping wild type. A fair parallel to hook in *D. melanogaster*, the latter being, however, a recessive.

orange (*or*).—April 1948, in four different wild chromosomes from Mogi, state of São Paulo. Eyes scarlet red (Ridgway, plate I) in young flies,

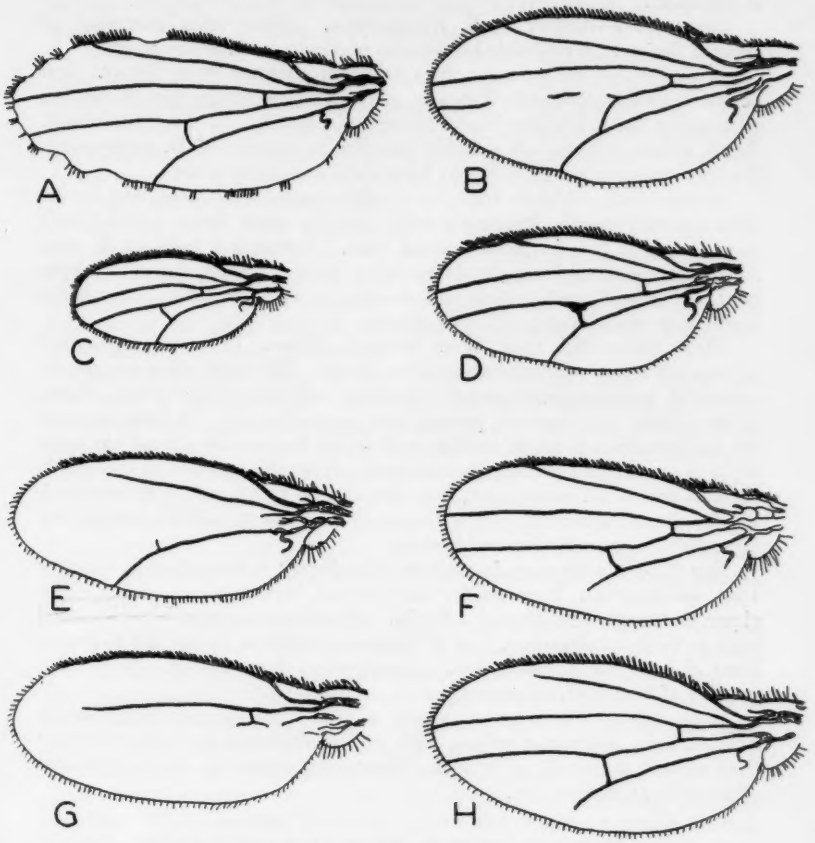


FIG. 1.—Some of the wing mutants in *Drosophila willistoni*. A—cut; B—Incomplete; C—miniature; D—Delta; E—Incomplete veinlet; F—short; G—reduced veins; H—Radius Incompletus. Magnification is similar in all figures.

darkening with age so that classification uncertain in old flies. A probable parallel to cinnabar in *D. melanogaster* and orange in *D. pseudoobscura* and *D. persimilis*.

pink-wing (*pw*).—August 1945, spontaneous. Eyes grenadine-red (Ridgway, plate II), age change slight. Body smaller than normal, wing somewhat shortened, wavy or crumpled, wing veins thickened and sometimes plexate. Thorax and abdomen reduced in size relatively more than the

head. The rows of acrostichal bristles near the middle of the thorax approximated and somewhat disarranged, the outer rows more widely spaced. Viability rather low. Testis sheath transparent. Homozygous females sterile. The remarkable manifold effect of this mutant resembles, judging from description, that of pink-wing in *D. melanogaster*, but homology is uncertain.

plexus (*px*).—August 1946, spontaneous. Wing veins branched or plexate (fig. 1). A probable homologue of plexus in *D. melanogaster*.

purple (*pr*).—October 1949, in a wild chromosome from Goyaz. Eye colour dragon's blood red (Ridgway, plate XIII) in young flies, darkening with age to about Madder brown (Ridgway, plate XIII). Ocelli coloured. Testis sheath yellow. A possible parallel to purple of *D. melanogaster*. Possibly the same as red of Ferry, Lancefield and Metz (1923).

scabrous (*sca*).—March 1948, in a wild chromosome from São Paulo. Eyes strongly rough, frequently with bulging giant facets which result from fusion of two or more normal ones. Acrostichal hairs in 8 rows, the outer rows containing the dorsocentral bristles. Some thoracal bristles bent or reduplicated. Although seemingly not identical, a probable homologue of scabrous in *D. melanogaster*.

Shaven (*Sv*).—May 1945, in an X-rayed culture. In homozygotes most bristles and many microchaetæ small or absent. The basal rings are usually preserved, and sometimes contain a hair-like rudiment of the bristle. Some of the bristles are, however, present and normal in size. In heterozygotes the manifestation is much weaker, only a few bristles are absent but most of them shorter and thinner than normal. Resembles shaven in the fourth chromosome of *D. melanogaster*, but may also be compared to abbreviated, Bristle, chaetelle, and reduced in the second chromosome of the same species. Homology is, accordingly, ambiguous.

Star (*S*).—March 1945, in an X-rayed culture ; a recurrence in October 1945, spontaneous. Eyes smaller than normal, surface rough, with a slight gleam as though covered with a liquid. The first occurrence was associated with a Y-II translocation, but a crossover obtained in an XXY female freed *S* from the translocation. Lethal when homozygous. A possible parallel of Star in *D. melanogaster*.

straw (*stw*).—September 1949, in a wild chromosome from Goyaz. Bristles, hair, and wings yellow, body colour yellowish but not as distinct from normal as that of the bristles. Penetrance complete. A good parallel of straw in *D. melanogaster*.

5. MUTANTS OF THE THIRD LINKAGE GROUP

aristapedia (*ss*^{a}).—May 1944, in an X-rayed strain of Rio de Janeiro origin. Recurrence in January 1948, in a strain of Belem origin, spontaneous. Aristæ transformed into leg-like organs. Variable, often asymmetrical, and overlaps wild type. The Rio allele is more penetrant than the Belem one, and behaves in heterozygotes with Delta as though the latter were a deficiency for *ss*^{a}. Rio homozygotes sterile as males. An excellent parallel to aristapedia in *D. melanogaster*.

bithorax (*bx*).—July 1946, spontaneous. Metathorax with bristles and hairs resembling those on the mesothorax. A probable homologue of bithorax or bithoraxoid in *D. melanogaster*.

brief (*bf*).—September 1949, in a wild chromosome of Goyaz origin. Bristles and hairs thin and shorter than normal. Body slightly smaller. Females sterile, males fertile. A possible parallel to brief in *D. melanogaster*.

claret (*ca*).—September 1949, in a wild chromosome of Belem origin. Eyes grenadine red (Ridgway, plate II) in young flies, darkening with age to about dragon's blood red (Ridgway, plate XIII). Testis sheath yellow. A possible parallel to claret in *D. melanogaster*.

Delta (Δ).—April 1944, in an X-rayed culture, and several recurrences, some of the latter associated with chromosomal aberrations. This is one of the commonest mutant types obtained in X-ray treated flies. Wing veins, especially the second longitudinal one, thickened and expanded at the wing margin. Posterior crossveins thickened and expanded into deltas at the ends. Lethal when homozygous. An excellent parallel to Delta in *D. melanogaster* and in other species.

karmoisin (*kar*).—May 1949, in a wild chromosome from Cruzeiro do Sul, Acre. Eyes scarlet (Ridgway, plate I) in young flies, darkening with age to about normal colouration. A recurrence in June 1949, in a wild chromosome from Paraná. Homology ambiguous—among the mutants in the right limb of the third chromosome of *D. melanogaster*, karmoisin and cardinal are similar.

loboid (*ld*).—July 1945, in an X-rayed culture. Eyes rough and misshapen in various ways. Most often the eye is constricted or divided by a cleft beginning at the posterior margin and progressing towards the anterior one. Often the eyes are bulging or drawn out into outgrowths covered with bristles and resembling an antenna. Occasionally overlaps normal. A good parallel to loboid in *D. melanogaster*.

pink (*p*).—March 1948, in a wild chromosome of São Paulo origin. Eyes dragon's blood red (Ridgway, plate XIII) in young flies, approaching normal colouration in old ones. Testis sheath lighter than normal. Homology ambiguous.

Radius Incompletus (*Ri*).—May 1944, in an X-rayed culture. Second and fifth longitudinal veins fail to reach the margin (fig. 1). In compound with Delta, the effects of the two mutants almost cancel each other, the flies being usually identifiable as very weak Deltas. Homozygote seldom survives as a fly almost wholly devoid of bristles and hair and only the costa and the third longitudinal vein complete in the wing, other veins reduced to mere stubs. No clear analogues in *D. melanogaster*.

Roof (*Rf*).—July 1945, in an X-rayed culture. The wings are held roof-like. The scutellum distinctly longer and relatively narrower than normal. A doubtful parallel to roof in *D. melanogaster*. Males sterile.

tardigrade (*td*).—May 1945, in an X-rayed culture. Tarsi rudimentary or absent, tibiæ and femora somewhat shortened. Variable. The degree of reduction of the tarsi usually greatest on the front legs, on middle and hind legs tarsi usually present but malformed in various degrees. Resembles tardigrade and combined of Ferry, Lancefield and Metz (1923) but not identical with either. Resembles the sex-linked mutant chilblained-*b* in *D. melanogaster*.

6. LINKAGE MAPS

The chromosomal complement of all known species of the *willistoni* group, and of most species of the *saltans* group, consists of a pair of metacentric X- or Y-chromosomes (indistinguishable at metaphase),

TABLE 1

Summary of linkage data. Three point crosses

Nature of the cross	0	1	2	1, 2	Total
1 *f* 2 *w*^{e} 1 2 *pn*	181-188	46-47	67-98	16-11	654
w^e 1 2 *N* 1 *f* 2	234-396	131-55	163-160	15-19	1173
lz 1 2 *pn* 1 *N* 2	137-158	116-108	0-0	0-0	519
1 *f* 2 *w*^{e} 1 2 *pn*	181-188	47-46	67-98	16-11	654
1 *Sv* 2 *bw* 1 2 *S*	360-233	227-240	17-18	13-16	1124
bw 1 *Hk* 2 *S* 1 2	176-244	143-123	217-199	102-56	1260
bw 1 *abb* 2 *S* 1 2	220-340	121-121	73-68	256-228	1427
bw 1 *Hk* 2 *S* 1 2	57-130	59-37	65-80	22-14	464
△ 1 *td* 2 1 2 *Rf*	206-279	30-44	96-33	5-8	701
ld 1 *bx* 2 1 2 *Rf*	189-75	7-6	81-59	6-2	425
td 1 2 *Ri* 1 *Rf* 2	239-264	164-116	4-5	3-5	800
△ 1 2 1 *ld* 2 *bx*	690-474	0-0	4-4	0-0	1172

TABLE 2

Summary of linkage data. Four point crosses

Nature of the cross	0	1	2	3	1, 2	1, 3	2, 3	1, 2, 3	Total
1 2 *se* 3 *sc* 1 *Inc* 2 3 *ru*	140-102	49-49	9-11	53-45	6-3	27-23	2-1	0-0	520
1 2 *co* 3 *sc* 1 *Inc* 2 3 *ru*	54-146	99-10	0-0	27-84	0-0	30-29	0-0	0-0	479
lz 1 2 *pn* 3 1 *sc* 2 3 *Inc*	303-355	40-65	153-140	0-0	3-7	1-0	0-2	0-0	1069
1 *ec* 2 3 *w*^{e} 1 2 *pn* 3 *sh*	226-157	41-64	53-122	18-15	9-8	5-4	16-26	3-1	766
w^e 1 2 *sn* 3 *pn* 1 *y* 2 3	78-170	21-15	35-17	92-45	0-1	2-9	1-2	0-0	488
1 2 3 *ve* *Inc* 1 *se* 2 *ru* 3	217-83	8-36	47-68	8-4	0-2	3-1	0-0	0-0	477
w^e 1 2 *sc* 3 *ru* 1 *y* 2 3	123-82	10-21	1-0	61-52	0-1	2-5	0-1	0-0	359
1 *sn* 2 3 *w*^{e} 1 2 *pn* 3 *ru*	250-83	80-39	33-95	84-60	12-5	31-32	19-24	5-4	856
1 *abb* 2 *Sv* 3 *bw* 1 2 3 *S*	248-192	65-124	183-155	14-11	42-39	18-9	5-1	5-5	1116
1 *pw* 2 3 *S* *Hk* 1 2 *Sv* 3	66-177	74-81	5-1	6-16	0-2	6-9	0-0	0-0	443
1 2 3 *Ri* *ld* 1 *bx* 2 *Rf* 3	327-136	8-10	143-225	30-38	1-2	1-0	20-19	0-0	960

a pair of metacentric second, and a pair of acrocentric third chromosomes. The small, dot-like autosome characteristic of many *Drosophila* species is absent, and the salivary gland cells show only five long and no short strands (the contrary findings of Wharton, 1943, are probably inaccurate). The three linkage groups found among the mutants thus correspond to the three pairs of chromosomes. Cytogenetic study of some chromosomal aberrations (unpub.) showed that the second linkage group is carried in the metacentric autosome and the third in the acrocentric autosome.

TABLE 3

Summary of linkage data. Five point crosses

Interval	*1w*^{a}*2sc3rs4*	*1w*^{a}*2lz3pn4*	*1 2 3Inc4*	*1sc2Inc3 4*	*1w*^{a}*2pn3 4ru*
	m1 2 3 4sh	*m1 2 3 4sh*	*w*^{a}*1sc2ct3 4pn*	*w*^{a}*1 2 3pn4ru*	*m1 2 3sh4*
0	225-169	197-112	235-215	246-230	311-372
1	4-3	3-0	47-40	42-31	5-0
2	24-14	4-4	126-137	111-141	264-179
3	207-123	121-77	3-1	3-0	120-115
4	0-6	87-49	0-1	83-96	15-5
1, 2	0-0	0-1	13-9	4-8	0-1
1, 3	0-1	0-4	0-2	0-0	1-0
1, 4	0-0	1-4	0-2	11-6	2-2
2, 3	10-21	1-0	2-0	2-0	99-43
2, 4	4-0	0-0	1-0	40-44	12-5
3, 4	0-0	63-24	0-0	0-0	0-0
1, 2, 3	0-0	0-0	0-0	0-0	1-0
1, 2, 4	0-0	0-0	0-0	0-2	2-0
1, 3, 4	0-0	0-0	0-0	0-0	0-0
2, 3, 4	0-1	0-0	0-0	0-0	0-0
Total	812	752	834	1100	1554

The linkage data are summarised in tables 1-3, and linkage maps of the chromosomes constructed on the basis of these data are shown in fig. 2. In contrast to the high variability in crossover percentages found in different experiments on *D. prosaltans* (see the companion paper of Spassky, Zimmering and Dobzhansky), the linkage values in *D. willistoni* are reasonably constant. This makes the construction of the linkage maps easier than in the former species.

7. DISCUSSION

Our data confirm the conclusion of Lancefield and Metz (1922) and Sturtevant and Novitski (1941) that the two arms of the metacentric X-chromosome of *Drosophila willistoni* more or less correspond to the X-chromosome and to the left limb of the third chromosomes respectively in *D. melanogaster*. Indeed, the first fifty units of the linkage map of the X of *D. willistoni* (fig. 2) contain the mutants miniature, white, lozenge, yellow, scute, forked and singed, which parallel the similarly named sex-linked mutants in *D. melanogaster* (Bridges and Brehme, 1944) to such an extent that the assumption that they are

homologous is probable. The similarity between the mutants echinus, cut, prune and Notch in the two species is not so striking as in the cases named above. Echinus of *D. willistoni* is, in fact, more like echinoid in the second chromosome than the sex-linked echinus in

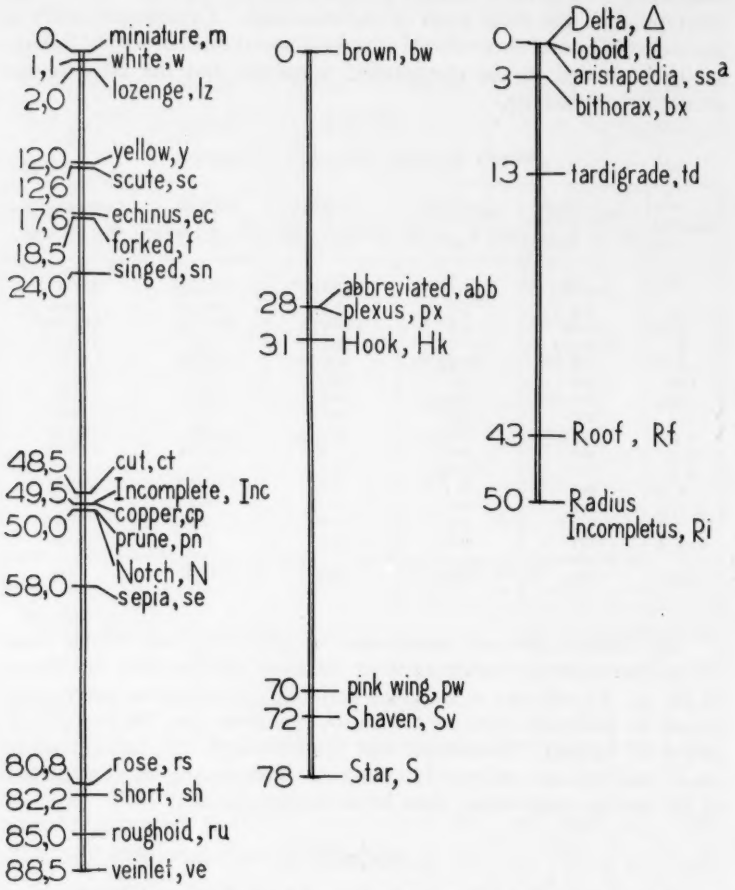


FIG. 2.—Linkage maps of the X-chromosome (left), second chromosome (middle), and third chromosome (right) of *Drosophila willistoni*.

D. melanogaster. Nevertheless, the assumption of homology is still the simplest one. The remainder of the linkage map (loci from sepia to veinlet) of the X-chromosome of *D. willistoni* contains sepia, roughoid and veinlet, all of which more or less resemble mutants in the left limb of chromosome III of *D. melanogaster*, and rose and short, the homology of which is obscure. Only the mutant stubbloid suggests a

homology with the right limb of the third chromosome of *D. melanogaster;* its location on the map of the X of *D. willistoni* is unknown since it has been obtained in a strain from Southern Brazil which gives no crossing over in the X-chromosome in hybrids with the Belem strain. It may be that a small translocation has taken place in the phylogeny of the species, or the mutants may be " mimics " rather than homologous.

The second chromosome of *D. willistoni* contains mostly the same genes as the second chromosomes of *D. melanogaster* and *D. prosaltans*, and the third plus the fourth chromosomes of *D. pseudoobscura*. This is suggested especially by the mutants brown, Emarginate, Hook, orange, plexus, scabrous and straw. The mutants pink-wing, purple and Star are less conclusive but still compatible with the above interpretation. Abbreviated and Shaven suggest, however, genes in the right limb of the third and in the fourth chromosomes respectively of *D. melanogaster*. Whether small translocations or " mimics " are involved, is an open question.

The third chromosome of *D. willistoni* should, then, be homologous mainly to the right limb of the third chromosome in *D. melanogaster*, and to the second chromosome of *D. pseudoobscura*. The best evidence of this comes from the mutants aristapedia, bithorax, Delta and loboid. The mutants claret, karmoisin, and pink are compatible with this interpretation. Radius Incompletus, Roof and tardigrade are ambiguous.

Comparison of *D. willistoni* with *D. prosaltans* shows that the distribution of genes among the chromosomes is similar in the two species: the X, the second, and the third linkage groups of the former correspond to the X, the second, and the third chromosomes of the latter. Now, these species are representatives of two species groups (*willistoni* and *saltans* groups) of the subgenus *Sophophora* of the genus *Drosophila*. Both groups are characteristically Neotropical in their distribution (Patterson and Wheeler, 1949). Apart from *willistoni* and *prosaltans*, some twelve species of these groups have been examined cytologically, and all but one of them have the same metaphase chromosome group: two large metacentric and a acrocentric chromosome pair, one of the metacentrics being the X. It seems, then, that the three linkage groups have tended to preserve their identities in the evolution of the Neotropical *Sophophora*.

Two other species groups belonging to the subgenus *Sophophora* are the *melanogaster* group, which is characteristically Oriental, and the *obscura* group (including *affinis*), which is Holarctic in distribution (Patterson and Wheeler, 1949). A variety of metaphase chromosome patterns are known in these groups, none of them identical with that in the Neotropical forms. However, the same metacentric autosome seems to occur in the *willistoni*, *saltans* and *melanogaster* groups (corresponds to the second linkage group in *D. willistoni*, *D. prosaltans*, *D. melanogaster* and *D. simulans*, and to the third in *D. ananassæ* and

D. montium (see Sturtevant and Novitski, 1941, for further references). The large metacentric X-chromosome of the Neotropical *willistoni* and *saltans* groups does not occur in the *melanogaster* group, but it is found in a majority of species of the Holarctic *obscura* group (*D. pseudoobscura*, *D. persimilis*, *D. miranda*, probably all species related to *D. affinis*, but not in *D. subobscura* and some Old World species related to the last-named one).

Finally, a small dot-like autosome is present in many species of several subgenera of *Drosophila*. In the subgenus *Sophophora*, this autosome occurs in all species of the *obscura* group and in some of the *melanogaster* group. In the Neotropical *willistoni* and *saltans* groups it is never present, and our data suggest that it has become broken up and incorporated into other linkage groups. In *D. prosaltans* a part of it is included in the X-chromosome, but at least one locus appears to lie in the third chromosome. The situation in *D. willistoni* is unclear ; the second-chromosome mutant Shaven in this species is not a reliable homologue of the similarly named mutant in the dot-like chromosome in *D. melanogaster*.

Closely related species of *Drosophila*, and even varieties of the same species, may differ considerably in the distribution of genes among the linkage groups (Stone and Patterson, 1947 ; Ward, 1949). Nevertheless, the correlations pointed out above between chromosome structure, systematic position, and geographic distribution indicate that, in the subgenus *Sophophora* and particularly in its Neotropical representatives, chromosome fusion and fragmentation have been, on the whole, infrequent in the phylogeny. Despite the probable occurrence of small translocations, the linkage groups preserve their essential integrity for long periods of time.

The gene arrangement inside the chromosomes is, on the contrary, frequently altered by inversions. Comparison of the linkage maps of the X-chromosome of *D. willistoni* obtained by ourselves (fig. 2), and by Lancefield and Metz (1922) discloses appreciable differences. Their maps show the genes forked and singed (stubby) some 30 units apart at the end of the chromosome, while yellow and scute are in the middle portion of their linkage map ; we found forked and singed only 6 units apart, and yellow and scute between forked and the end of the map. These disagreements are almost certainly due to our mutants having been obtained in a strain of Brazilian origin, whereas Lancefield and Metz worked with a strain from Cuba. These strains probably differed by inversions.

The variability of the gene arrangement within a species may seem to make comparisons of different species meaningless. Actually some associations between neighbouring genes are quite persistent. Thus, yellow and scute are adjacent in species as diverse as *D. willistoni*, *D. prosaltans*, *D. pseudoobscura*, *D. persimilis*, *D. melanogaster*, *D. simulans*, *D. ananassæ* and *D. virilis*, although not in *D. affinis* and *D. hydei* (*cf.* Sturtevant and Novitski, 1941). On the other hand, white is

close to yellow and scute in *D. melanogaster* and *D. simulans*, somewhat farther apart in *D. willistoni* and *D. pseudoobscura*, and very far apart in *D. prosaltans*, *D. ananassæ* and *D. virilis*.

The length of the linkage map of a chromosome depends, in part, on the number of loci known in a linkage group. The more mutant loci are placed, the greater the probability that loci near the ends of the chromosome will be included, and the greater the chance that the total length of the linkage map will be known. Despite this source of error, comparison of the published linkage maps for different species of *Drosophila* suggests the existence of genetic differences in the amount of recombination in homologous chromosomes in these species. In species with large metacentric X-chromosomes, the known map lengths of the X are some 90 units in *D. willistoni*, 100 in *D. prosaltans*, 165 in *D. persimilis* and 180 in *D. pseudoobscura* (Beers, 1937 ; Sturtevant and Tan, 1937 ; Spassky, Zimmering and Dobzhansky, 1950, and the present article). In species with acrocentric or small metacentric X-chromosomes, the map length of the X is 66 units in *D. melanogaster* and *D. simulans*, 116 in *D. ananassæ*, 150 in *D. subobscura* and 170 in *D. virilis* (Bridges and Brehme, 1944 ; Chino, 1936 ; Moriwaki, 1938 ; Spurway, 1945). As stated above, only parts of the large metacentric X's correspond to the short metacentric or to acrocentric X-chromosomes. Approximate estimates of the length of these parts are 55 units in *D. willistoni*, between 60 and 70 in *D. prosaltans*, about 60 in *D. persimilis* and 73 in *D. pseudoobscura*.

The large metacentric autosome is at least 59 units long in *D. prosaltans*, 78 in *D. willistoni*, 94 in *D. ananassæ* and 108 in *D. melanogaster*. The combined lengths of the two acrocentric autosomes which correspond to the above metacentric ones are 137 units in *D. pseudoobscura* and 379 in *D. virilis*. Finally, the acrocentric autosome is 50 units long in *D. willistoni*, 52 in *D. prosaltans* and 210 in *D. virilis*. Comparison of these figures suggests the conjecture that small amounts of recombination are characteristic of tropical species, and high amounts of those distributed chiefly in the temperate zones (*D. willistoni*, *D. prosaltans* and *D. ananassæ* are tropical ; *D. melanogaster* and *D. simulans* now cosmopolitan but originally probably subtropical, while *D. pseudoobscura*, *D. persimilis* and *D. virilis* are temperate). More data are obviously necessary to test the validity of this conjecture.

8. SUMMARY

Drosophila willistoni has a large metacentric X-chromosome, a large metacentric second chromosome and a acrocentric third chromosome. Three linkage groups corresponding to these chromosomes are established.

Comparison of the mutants indicates that the chromosomes of *D. willistoni* contain largely the same genes as the cytologically similar chromosomes of *D. prosaltans*, although the sequences of these genes

within the chromosomes are quite different, because of the repeated occurrence of paracentric inversions in the phylogeny. Homologies with the chromosomes of other species of *Drosophila* are suggested. Certain correlations between the systematic position, geographic distribution, and the chromosome contents in species of the subgenus *Sophophora* of *Drosophila* appear possible. The two Neotropical sections of the subgenus, namely, the *willistoni* and the *saltans* species groups, have very similar chromosomes. The large metacentric X-chromosome characteristic of these sections appears also in most species of the Holarctic *obscura* species group, but not in the Oriental *melanogaster* species group. Conversely, the large metacentric autosome of the *willistoni* and *saltans* groups is shared with *melanogaster* but not with the *obscura* group. The acrocentric autosome of the *willistoni* and *saltans* groups occurs also in the *obscura* group, but in the *melanogaster* group it is joined with a chromosome which in the *willistoni*, *saltans*, and in most species of *obscura* group is a part of the X-chromosome.

The amounts of recombination which occur in corresponding chromosomes of different species vary greatly. A suggestion is made that tropical species have less recombination than species which inhabit temperate lands.

9. REFERENCES

BEERS, C. V. 1937.
Linkage groups in *Drosophila pseudoobscura* race B.
Genetics, *22*, 577-586.

BRIDGES, C. B., AND BREHME, K. S. 1944.
The mutants of *Drosophila melanogaster*.
Carnegie Inst. Washington, Publ. 552, 1-257.

CHINO, M. 1936.
The genetics of *Drosophila virilis*.
Jap. J. Genet., *12*, 187-210, 257-277.

FERRY, R. M., LANCEFIELD, R. C., AND METZ, C. W. 1923.
Additional mutant characters in *Drosophila willistoni*.
J. Heredity, *14*, 373-384.

LANCEFIELD, R. C., AND METZ, C. W. 1922.
The sex-linked group of mutant characters in *Drosophila willistoni*.
Amer. Nat., *56*, 211-241.

MORIWAKI, D. 1938.
The genetics of some mutant characters in *Drosophila ananassæ*.
Jap. J. Genet., *14*, 1-22.

PATTERSON, J. T., AND WHEELER, M. R. 1949.
Catalogue of described species belonging to the genus Drosophila, with observations on their geographical distribution.
Univ. Texas Publ., *4920*, 207-233.

RIDGWAY, R. 1912.
Color standards and color nomenclature. Washington.

SPURWAY, H. 1945.
The genetics and cytology of *Drosophila subobscura*. I. Element A.
J. Genet., *46*, 268-286.

SPASSKY, B., ZIMMERING, S., AND DOBZHANSKY, TH. 1950.
Comparative genetics of *Drosophila prosaltans*.
Heredity, *4*, 189-200.

STONE, W. S., AND PATTERSON, J. T. 1947.
The species relationships in the virilis group.
Univ. Texas Publ., *4720*, 157-160.

STURTEVANT, A. H., AND NOVITSKI, E. 1941.
The homologies of the chromosome elements in the genus Drosophila.
Genetics, *26*, 517-541.

STURTEVANT, A. H., AND TAN, C. C. 1937.
The comparative genetics of Drosophila pseudoobscura and D. melanogaster.
J. Genet., *34*, 415-432.

WARD, C. L. 1949.
Karyotype variation in Drosophila.
Univ. Texas Publ., *4920*, 70-79.

WHARTON, L. T. 1943.
Analysis of the metaphase and salivary chromosome morphology within the genus Drosophila.
Univ. Texas Publ., *4313*, 282-327.

HYBRIDITY SELECTION IN *CAMPANULA*

C. D. DARLINGTON and L. F. LA COUR
John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.

Received 25.viii.49

CONTENTS

1. Introduction 217
2. Building New Types 218
3. Ring-Formation and the Hybridity Optimum . . 219
4. Analytic Crosses 222
5. Unbalanced Types 224
6. Fertility and the Breeding System 226
7. New Interchanges 227
8. The Stable Telocentric 228
9. Telocentrics and Interchanges 230
10. A Monosomic Plant 231
11. New Telocentrics and Isochromosomes . . . 233
12. The Origin of Isochromosomes 237
13. Pollen-Grain Inheritance 239
14. The Future of Isochromosomes 240
15. The Evolution of Chromosome Numbers . . . 241
16. Conclusions 242
17. Summary 243
18. References 246
Appendix: Compound Constrictions by L. F. La Cour 243

1. INTRODUCTION

In our first account of ring formation in *Campanula persicifolia* (Gairdner and Darlington, 1931) we showed how mechanical rules could be applied for the construction of plants with large rings at meiosis by the combination of interchanged chromosomes found in wild races. In our second account (Darlington and Gairdner, 1937) we showed how large rings were maintained by the elimination of homozygotes but broke down more frequently than those stabilised by natural selection in *Oenothera*, owing to the occurrence of crossing-over between interstitial segments in opposite complexes. We also showed the high degree of structural hybridity in regard to inversions which was correlated with interchange hybridity in a cross-fertilising species. Our present object is to make use of the interchanges and other breakages as markers in determining how hybridity is maintained by selection in the species, in other words how the breeding system works. In this programme the origin, maintenance and breakdown of telocentric chromosomes plays a part.

We are indebted to Dr Margaret Upcott for the crossings, scorings, drawings, photographs and fertility records from 1937 to 1939.

The earlier preparations of pollen mother-cells were aceto-carmine and Feulgen smears, the later acetic lacmoid. Feulgen alone was suitable for the pollen-grain mitosis owing to the thickness of the walls.

Symbols.—The fraction of the circle in black used in the tables represents the fraction of the 16 chromosomes in the ring or rings. Pairs of single lines outside the circle represent pairs of telocentrics, opposite pairs represent telocentric bivalents; pairs of joined lines, isochromosomes; attachment to rings or to free chromosomes is indicated by position. *t*, telocentric; *iso*, isochromosomes. *B*, Basic type, the common original type found generally in Europe in the absence of interchange. *R*, reciprocal crosses.

2. BUILDING NEW TYPES

In the first place it is necessary to record the continuation of the work of building new types of structural heterozygotes by crossing individuals carrying different interchanges. The products of such

TABLE 1

Synopsis of families since 1937

Recombination progenies from crossing dissimilar parents
R: include reciprocal crosses

	Build-up types								Breakdown types			Total
Parents	⊙	◔	◓	◔	◓	◑	◑	◒	◓	◓	◓	–
◓ × ◓	2	–	–	–	–	–	–	–	–	–	1	3
◒ × ◓ ex ◒	2	1	4	2	2	5	3	–	–	1*	–	20
◓† × ◒	–	–	1	–	–	2	2	–	8	–	–	13
◓ × ◓	–	1	1	2	3	1	2	–	–	–	1	11
◑ × ◑	1	–	–	–	–	–	1	–	–	–	–	2
◑ × ◓ *R*	4	1	1	2	6	8	11	–	–	–	–	33
◑ × ◒	–	7	–	17	–	9	5	1	–	–	–	39
◒ × ◑ *R*	2	1	–	2	–	4	–	5	–	–	–	14
◒ × ◒ *R*	1	2	2	1	–	–	3	2	–	–	–	11
Total	12	13	9	26	11	29	27	8	8	1	2	146

* Due to new interchange in one parent
† Russian

synthetic crosses consist of old types, and new ones with larger rings; but they also include what we may call *breakdown* types in which the rings formed represent a partial disintegration of a ring-of-twelve, *i.e.* 6+6, 8+4, and 4+4+4 (Table 1).

3. RING-FORMATION AND THE HYBRIDITY OPTIMUM

The results of selfing and inter-crossing similar ring types of plant are, apart from unbalanced forms, of three kinds :—

(i) *Structural heterozygotes* with the parental type of ring.

(ii) *Structural homozygotes* with eight bivalents : they are homozygous for the structural arrangements of one of the two balanced complexes produced by segregation in a ring.

(iii) *Intermediates* with smaller or fewer rings : these last are the result, either of crossing-over or of non-disjunction within the ring of the kind which gives half-mutants in *Oenothera*, or again of homozygosis for one ring where the parents had two.

In order to interpret our results we have to consider what differences are to be expected between the structural heterozygotes and homozygotes in selfs and also in crosses. For this purpose the chromosomes

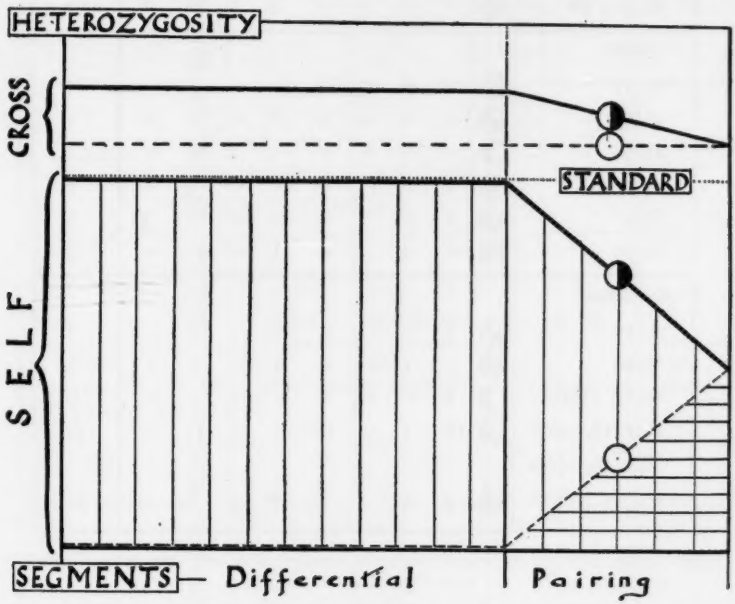


FIG. 1.—Graph showing comparative heterozygosity of selfed and crossed plants in respect of the pairing and differential segments of chromosomes derived from a ring (*e.g.* a ring-of-eight), the ring parent being the standard.

in ring parents consist of two parts, the *differential segments* which cannot cross over with their homologues, and the *pairing segments* which can and do cross over. So far as any particular plant is concerned what matters is the position of the chiasmata in its parental mother-cells.

But in a sample of plants the pairing segments will show a gradient from no crossing-over next to the differential segments to 50 per cent. crossing-over at the ends.

Now take the progeny from *selfing* a ring plant, structurally homozygous (non-ring) and heterozygous (ring). The non-rings will be genically homozygous for their differential segments and have a

TABLE 2

Synopsis of families since 1937 D. and G. table III

Maintenance progenies, from similar parents, 150 seedlings

Type of ring	⊙	◔	◓	◑	◕	◑	◑	◕	Total
Selfs									
15/37	–	1	–	4	7	–	–	–	12
12, 13/38	–	–	2	2^{1}	3	5	–	–	12
9/39, 4/40	–	–	3	–	4	1	2	2	12
Sisters									
23/37	2	5	7	–	–	–	–	–	14
27/39	–	–	–	–	1	–	–	–	1
24/38	–	–	–	–	–	1	–	–	1
14-17/38	2	1	–	–	–	–	5	–	8
8/39	1	–	1	–	–	–	–	3	5
19/39	–	1	1	–	1	–	–	–	3
Non-sisters									
20/40	1	–	–	–	–	–	–	–	1
19/40	–	1	–	–	1	–	–	–	2
19/37, 12/40	3	–	–	–	–	3	–	–	6
1/37, 18, 19/38	12	1	1	10^{2}	–	3	15	–	42
8, 9/40, 14/42									
1-7/39, 5, 6/40	3	4	1	6	2^{1}	4	4	7	31^{1}

NOTE.—Index numbers on progeny show the numbers that were trisomic and therefore of non-disjunctional origin.

genic heterozygosity rising in a gradient in the pairing segments towards half the parental degree at the ends. The rings will be genically as heterozygous as the parent was for the differential segments and have a genic heterozygosity diminishing in the pairing segments towards half the parental amount at the ends. In other words rings and non-rings will be sharply differentiated in genic as well as in structural heterozygosity.

TABLE 3

Progenies of similar parents according to their relationships (total data before and since 1937)

(i) SINGLE RINGS (lower rings are due to breakdown by crossing-over or non-disjunction)
(ii) DOUBLE RINGS (single rings are due to recombination)

Parents	Offspring			
	⊙	Lower ring or single ring	Parent type	Total
(i) SINGLE RINGS				
Self ◔	–	4	1	35
◑	–	1	5	
◑	–	12	–	
◕	–	10	2	
	0	27	8	
	0·0%	77·1%	22·9%	
Sister ◔	–	–	7	50
◑	10	2	17	
◑	2	1	5	
◕	2	1	3	
	14	4	32	
	28·0%	8·0%	64·0%	
Non-sister ◑	3	–	3	79
◑	12	15	16	
◕	3	20	7	
	18	35	26	
	22·7%	44·4%	32·9%	
Total	32	66	66	164
(ii) DOUBLE RINGS				
Self ◓	–	1	2	3
◕	–	5	7	12
Sisters ◓	2	5	7	14
◕	–	–	1	1
◕	–	3	–	3
Non-sisters ◓	1	–	–	1
◕	–	1	1	2
Total (1 : 2 : 1)	3	15	18	36

The progeny from crossing with plants having similar rings but dissimilar ancestry will not be distinguished in anything like the same degree. The structural homozygotes can be genically highly heterozygous and the structural heterozygotes need be little more so. Again, in the pairing segments, what difference there is will decrease towards the ends. The points of interchanges, as indeed of inversions, are thus markers of ancestry and differentiation. But since the interchanges (in *Campanula*) can be taken to pieces and put together again, they have to be considered more than are inversions, together with the known relationships of chromosomes and plants (fig. 1, p. 219).

If there is a differential elimination of genic homozygotes in mixed progenies the proportion of structural homozygotes and heterozygotes should therefore differ with the relations of the parents. Structural homozygotes should be fewest (and rings commonest) in the selfed progeny, more frequent in sister and non-sister progeny. This is in fact the case for both single and double rings (tables 2 and 3). The use of the interchanges as markers thus enables us to prove the existence of a *hybridity optimum* which is favoured by selection. The non-rings are eliminated in selfs since they depart in character from the naturally selected products of the breeding habit of the species. But they are not eliminated in crosses since here they conform in character with those naturally selected products.

It will be noted, on the other hand, that low rings from high rings (table 2(i), due to crossing-over) are favoured in selfs and non-sister crosses but that single rings from double rings (table 2(ii), due to recombination of the pre-existing types) are not so favoured. This would indicate that certain low rings were favoured in certain crosses, not because they gave the maximum gene-hybridity, but because they constitute appropriately balanced combinations. It is a question of external balance being more important than internal balance (to use Mather's, 1942, distinction) in particular combinations. Catcheside (1936) has arrived at the same conclusion in *Oenothera* by showing that the addition of different extra chromosomes will render a particular lethal diploid combination viable.

4. ANALYTIC CROSSES

In building multiple from single interchange rings the interchanges are not likely to be all on the same side of the ring. In our high rings both sides of the ring and both gametic types should therefore contain interchanges with respect to our original basic type. When they are crossed with basic types all the progeny will therefore be expected to show rings, chiefly rings-of-four. This is borne out by the results of our analytic crosses. Apart from one presumed mistake, all the progeny have rings, most of them two rings-of-four (table 4).

The non-ring or structurally homozygous progeny from selfing high rings must therefore be interchange homozygotes. We might expect

that those from rings-of-eight, -ten and -twelve would usually give two rings-of-four when crossed with the basic type. In fact in all five test families they uniformly did so (table 5). It seems likely therefore that this combination of interchanges is the only one in our high-ring stocks which is viable as a homozygote. One apparent exception to this rule was manifestly spurious and due to a new interchange (section 8).

TABLE 4

Synopsis of families since 1937

Analytic crosses, *i.e.* between high rings and basic homozygotes (112 seedlings)

Families	Ring parent	⊙	◔	◕	◑	◕	◑	Total
(1937) . .	◑	–	14	–	–	–	–	14
(1937 *et seq.*) .	◑	–	6	15	–	–	–	21
20, 21/38 and 48/39 ; 10/42	◑	–	3*	20†	–	2	–	25
7-10/38 . .	◕	–	–	32	–	12	2‡	46
21/48, 9/42 .	◑	(1)§	2	2	1	1	–	6
Total		–	25	69	1	15	2	112

* Due to breakdown by crossing-over.
† One trisomic.
‡ Due to new interchange in parent : weak plants.
§ Presumed mistake.

TABLE 5

Synopsis of families since 1937

Test crosses between basic (B) and extracted homozygotes

Parents	No. of families	Progeny
⊙ ex ◑ × ⊙ B	2	3 ◕
⊙ B × ⊙ ex ◑	2	5 ◕
⊙ ex ◕ × ⊙ B	1	2 ◕

Thus single interchanges and certain combinations of interchanges are lethal and, therefore, capable in themselves of maintaining the ring in *Campanula*, making it breed true by a balanced lethal system in nature. Certain combinations of interchanges, however, are not lethal in the homozygous condition. One interchange, or at least one interchanged chromosome, sometimes compensates for the effects of another. This point was also proved by the segregation of non-basic homozygotes from the Russian stocks dealt with later.

5. UNBALANCED TYPES

Non-disjunction increases in frequency with the size of the ring. It was numerically unequal in 29 per cent. of pollen mother-cells with a ring-of-eight, in 50 per cent. with a ring-of-ten. Trisomics, therefore, have appeared in the progeny of plants with rings-of-eight, -ten or -twelve but not with smaller rings. The extra chromosome which is sometimes lost somatically, can be transmitted through the pollen as well as through the eggs (table 6). Even when the trisomic is used as the mother, however, the trisomic progeny is less frequent than the disomic and is evidently less viable (table 7).

TABLE 6

Origin of trisomics

Female parent	Male parent	No. of progeny	Trisomics
◑	◕	*35*	2
	⊙	*7*	1
◑	◕	*33*	3
	◕	*7*	1
⊙	◕	*12*	1
Total		*94*	8

NOTE.—Since no trisomics have been found in the progeny of non-rings or even lower rings than eight, the extra chromosome from a basic × (12) + 2(2) must be derived from a ring of the *father*. It is attached to one of the two rings-of-four in the offspring.

TABLE 7

Progenies showing partial elimination of the extra chromosome of a trisomic in transmission on the female side

Families 2/ and 8/37 : ◕ (4+1)+(6)+3(2) × ◑ (10)+3(2)						
	⊙	◔	◑	◕	◕	Total
$2x$ . . .	4	4	2	2	1	13
$2x+1$ . .	—	*1*	—	*2*	*2*	*5*

The trisomic plants have configurations at meiosis of the types classified by Catcheside (1936) according to the kind of non-disjunction by which they arise (fig. 2). They have in addition aberrant configurations, some due to the pairing of the reduplicated distal segments previously described, and some presumably due to the formation of ring bivalents with chiasmata proximal to a small terminal interchanged segment (fig. 3). In our progeny records the

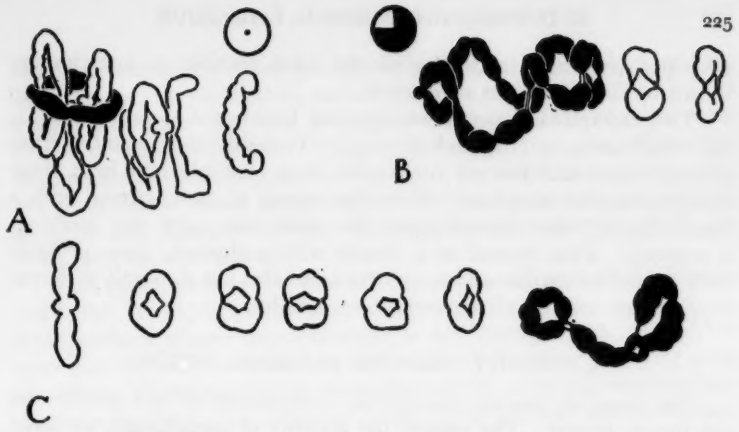


FIG. 2.—First metaphase of meiosis in the pollen mother-cells of *Campanula persicifolia*.

A Interlocking in Russian plant (Valdai) with 8 bivalents ($49^{22}/37$).

B. Ring-of-twelve in $6^{8}/38$.

C. Trisomic plant with extra chromosome joining 2 bivalents ($2^{12}/37$). Figs. 2 to 11 magnified ×2700. The symbols in the figures illustrate those used in the tables.

FIG. 3.—Various configurations in a trisomic plant with rings-of-two and -four joined by a chain-of-three. The black bivalents in C, D and E are derived from the chain-of-nine in B. One of these in D and E is not a true bivalent; it has an interchanged end distal to the interstitial chiasma.

trisomics are scored according to the total number of interchanges shown by the maximum association.

Two tetraploids arose in crosses, one between 2(4) and (6)+(4), the other between (10) and (6)+(4). Probably they resulted from gametic non-reduction on both sides since multiple and high rings hinder a regular anaphase. From the second of these, crossed with a basic diploid, one capsule gave 200 seeds but only one seedling, a triploid. This, crossed as a female with a diploid, gave progeny with 19 and 20 chromosomes, in form and habit not distinctly different from a sister with 16 chromosomes but smaller.

6. FERTILITY AND THE BREEDING SYSTEM

The previous account showed the dependence of seed fertility on the female parent. The greater the number of interchanges involved in its ring the lower its fertility. The present extended data show more (table 8). Now it is clear that the male parent, at least with

TABLE 8

Fertility summary (including table 7 of D. and G. 1937)

Percentages of potential maximum of 600 seedlings per capsule produced in various crosses showing correlation with the number of interchanges in the female, but not in the male, parent.

Number of interchanges	0	1	2	2	3	3	4	5	Average
♀ \ ♂	⊙	◔	◒	◑	◕	◑	◕	●	
⊙	30	24	–	27	–	21	20	21	24·0%
◔	27	11	–	–	–	–	–	–	19·0%
◒	–	–	9	–	–	1·5	5	–	5·5%
◑	12	–	–	14	–	–	–	–	13·0%
◕	–	–	–	–	13	–	–	–	13·0%
◑	3·5	–	–	–	1·3	2·3	4·0	3·3	2·9%
◕	6·0	–	–	–	1·5	–	2·0	2·0	2·9%
●	2·5	–	–	–	1·6	1·0	1·5	0·5	1·4%

artificial and therefore excessive pollination, has little or no influence on seed fertility. Thus pollen which can fertilise the egg is equally able in all classes to produce viable embryos. And unbalance, produced by excess or deficiency of whole chromosomes, is eliminated largely or entirely in the haploid stages of pollen and embryo-sac.

The irregularity and infertility of large rings produced experimentally in *Campanula* may be contrasted with the fertility of the large natural rings of *Oenothera*, *Rhoeo*, and *Hypericum*. In these regularity must have been achieved as a result of a slow process of

selection during the development of the ring. This development has been traced in parallel in several sections of *Oenothera* by Cleland (1944) who finds that it is accompanied by a change of habit from cross- to self-fertilisation. We can now see from the differential elimination of homozygotes in *Campanula*, described in section 3, that the transition is bound to favour the selective survival of ring-forming progeny and hence determine the evolutionary sequence which has been inferred in *Oenothera*.

The conclusion to be derived from the differential elimination may also be expressed in general terms to cover the evolutionary consequences where ring-formation is not brought into play. We may say that *wherever there is a rise or fall in the size of a mating group the degree of heterozygosity of the population will not rise or fall correspondingly without a time lag*. The breeding system is self-buffered against change.

7. NEW INTERCHANGES

Additional interchanges, enabling us to increase the ring still further, have been found in Russia. Rings-of-four occur in seed from three new localities (table 9*a*). Crosses again showed that all the

TABLE 9*a*

Origin of new interchanges and telocentric fragments. Seed collected 1937 : 72 seedlings

Collector	Locality	Symbol	Samples		
			⊙*	◔	⊙
H. G. Tedd (through W. B. Turrill)	Xanthi (Thrace)	–	14	0	0
H. N. Barber . .	Stolzenfels (Coblenz)	Stz	10	1	0
,, . .	Heidelberg	–	3	0	0
Pojarkova . . .	Oranienbaum, near Leningrad	Or	17	5	0
Savicz . . .	Valdai Hills	Val †	20	1	1

* Proved to be basic by crossing with basic types and *inter se*.
† One of each of the three types had a new character " dark pollen."

rings depended on combinations of single interchanges with the basic type : it was common to western and eastern Europe. They also showed that two of the Oranienbaum interchanges were different and both were different from the Valdai interchanges (table 9*b*). It seems that on its eastern frontier the species becomes more tenacious of its chromosome variability. This variability is also shown in a new way now to be described.

TABLE 9*b*

Relationships of new interchanges (29 seedlings)

Parents			Progeny			
			⊙	◔	◕	◑
Or	⊙ × Stz	◔	1	1	–	–
Or^{1}	◔ × Stu	⊙	2	3	–	–
Or^{26}	◔ × Or^{9}	◔	2	–	–	–
Or^{26}	◔ × Or^{14}	◔	–	4	–	–
Or^{9}	◔ × Or^{14}	◔	–	2	1	–
Or$^{9, 19}$	◔ × Val	◔	3	1	–	2
Or$^{14, 26}$	◔ × Val	◔	3	4	–	–
Or^{9} × Or^{11}	◕ × Or Val	◑	–	–	1	–

8. THE STABLE TELOCENTRIC

One of the Valdai plants had seven-ring bivalents and a chain-of-three (fig. 4A). Two small telocentric chromosomes were united with the two arms of an ordinary chromosome. Each was equal in

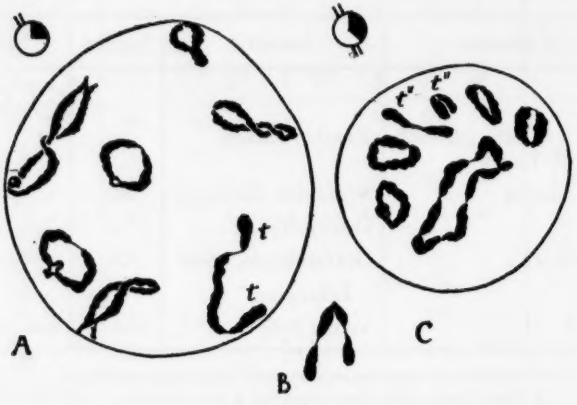


FIG. 4

A. Heterozygous telocentric triad with ring-of-four at late diplotene.
B. Telocentric triad at metaphase : each telocentric equal to the arm it is paired with.
C. Homozygous telocentric with ring-of-six at diakinesis. Feulgen preparations.

length to the arm with which it was paired. The two were therefore complementary and had no doubt arisen by complementary misdivision in a pair of homologous univalents (fig. 12). Disjunction at first anaphase was completely regular.

This heterozygous telocentric plant, for such we may call it, provided a marker for the unbroken chromosome partner and especially for its hybridity. Wide crosses indicated by their normal ratios an equal survival of all types; and inbred families indicated by abnormal ratios an elimination of homozygotes, either telocentric or of normal mesocentric type. The principle of the hybridity optimum, demonstrated by the interchanges, was confirmed (table 10).

TABLE 10

Inheritance of original telocentrics without reference to the rings (145 seedlings) (see also table 12)

	Family	Cross	Progeny ⊙ ⊙ ⊙	Total
Russian	Val[27] selfed	⊙ *s*	2 3 0	5
Russian × Western (*R*)	Val[27] × Stu	⊙ × ⊙ (*R*)	10 5 –	45
	48/39, 10/41 ; 14-16/42	⊙ × ⊙ (*R*)	14 16 –	
Russian × Russian	17-23/42*	⊙ × ⊙	1 30 –	53
	17-23/42*	⊙ × ⊙	2 20 –	
	4, 5/41 ; 8-10/42	⊙ × ⊙ (*R*)	– 31 –	31
Mixed	7-9/41	⊙ × ⊙ (*R*)	– 9 2	11

* In this cross between Russian stocks, and its reciprocal, the non-telocentric progeny were mostly eliminated. Evidently the telocentric heterozygotes had an advantage over the normal homozygotes (see also table 11).

Both heterozygous and homozygous telocentrics were stable. A new type of *Campanula persicifolia* with two pairs of telocentrics, making 18 chromosomes in all, $n = 9$, was produced in the next generation in families 1/40 and 3/40 (fig. 4C).

The telocentrics which have maintained themselves in nature are thus, as we should expect, more stable than those that have arisen in experiments in *Zea* and *Fritillaria* (Darlington, 1940*a*). Nevertheless they are genuinely telocentric, unlike many similar chromosomes in the Orthoptera, for they do not form chiasmata in any short arm. This is significant for they have been seen twice to form chiasmata very close to the centromere in their single long arm. Such chiasmata show, by the way, that in *Campanula*, as has been assumed elsewhere (Darlington, 1940*b*), localisation favours chiasmata *near* the ends even more than it favours them *far* from the centromeres. Localisation is distal as opposed to proximal. It is also terminal as opposed to intercalary. And the two oppositions are balanced.

9. TELOCENTRICS AND INTERCHANGES

Crosses of the telocentric stocks made between our original rings-of-ten and -twelve and derivatives from the Russian stocks brought the telocentrics into the ring (table 11).

TABLE 11

Combination of telocentrics and interchanges showing the origin of chains of 5, 7, 11 and 15 chromosomes (26 seedlings)

Family	Cross	Progeny types	Total
8/42	⊙ × ● (Or × ◔)	1 ⊙ 1 ◔ 1 ◕	3
15, 16/42	◕ × ◔ (Or × ◕) ex ●	2 ◕ 1 ◔ – 1 ◑ 1 ◔ 1 ●*	6
22, 23/42	⊙ × ◕ (*R*) ($13^{10}/41$)	1 ◑ 1 ◔ – 2 ◔ 2 ◔ 8 ◕ 1 ◕ 1 ● 1 ●	17

* Chain-of-fifteen.

The effect of the telocentrics is, of course, to break the ring into a chain, in one case a chain-of-fifteen chromosomes leaving out only one bivalent ($15^{16}/42$). The interest of these configurations is that they simulate the heterozygous conditions of sex chromosomes which are inherently self-perpetuating both in plants and animals (fig. 5). The

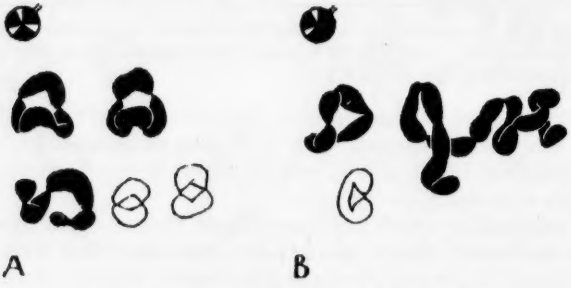


FIG. 5.—Telocentrics introduced into the ring.
A. In the third ring-of-four making a chain-of-five.
B. In the ring-of-ten making a chain-of-eleven.

experiment shows the simple steps by which it has long been assumed that these systems are built up (Darlington, 1932, 1937).

The analogy here is different from that with *Oenothera*. Species with sex chromosomes are, like *Campanula*, normally cross-fertilising and therefore, like *Campanula*—with rare and unstable exceptions—develop only single-interchange heterozygotes in nature.

10. A MONOSOMIC PLANT

In a cross between sister heterozygous telocentrics (table 12*a*) a new type of plant appeared ($1^{12}/40$). Externally it seemed normal apart from certain light green mutant sectors on the leaves. It had 15 chromosomes and may be represented as of the constitution $2(2)+3(4)-1$, that is to say the missing chromosome was a member of what should have been a third ring-of-four (fig. 6). Now, since

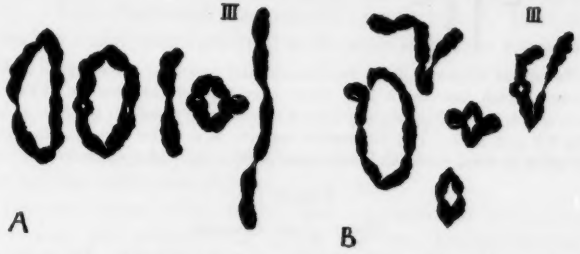


FIG. 6.—Two cells of the monosomic plant with three potential rings-of-four $1^{12}/40$.

three rings-of-four cannot be extracted from the ring-of-ten of the sole interchanged grandparent, the third ring must be due to a new interchange. The origin of a new interchange by crossing-over or an equivalent process at meiosis (*cf.* Darlington, 1931 ; fig. 27) would itself favour the three-to-one disjunction responsible for the monosomic. The two accidents must therefore be correlated and the plant derived from an $n-1$ gamete.

Earlier monosomic plants had arisen by evasion of the $n-1$ generation, as with parthenogenesis in *Oenothera* (Stomps, 1931), with somatic mutation in many plants, or with X-rayed pollen (Stadler, 1931) which may well have lost a broken chromosome by sister reunion of its chromatids after fertilisation.

Our monosomic resembles two more recent cases, those in *Godetia* (Håkansson, 1945) and the grasshopper *Chorthippus* (Coleman, 1947) in two respects : (i) it has arisen from an $n-1$ gamete ; (ii) the missing chromosome is not a member of the standard complement but a new chromosome produced by interchange and probably of reduced length. It is clear that interchange followed by monosomy provides the opportunity for endless trials in nature of the viability of deficiencies. It was suggested (Darlington, 1939 ; fig. 19) that such interchanges might be the basis of reduction of chromosome number through loss of the new short chromosomes.

This process has now been confirmed by Håkansson's discovery (1946) of nullisomics in *Godetia Whitneyi* where the basic number is reduced from 7 to 6. The precise pachytene relationships are not clear in *Godetia* but in *Chorthippus* Coleman's account enables us to reconstruct them exactly (fig. 7). They show that in fact a new

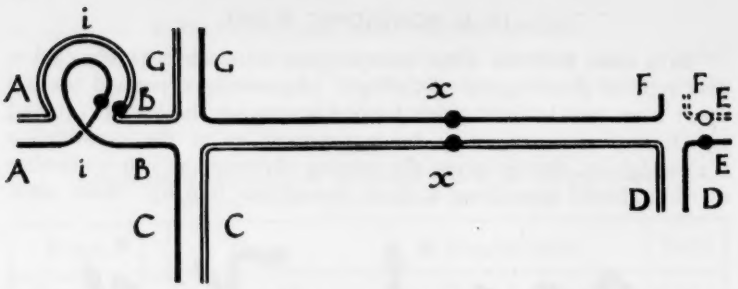


FIG. 7.—Pachytene diagram of the interchange and inversion heterozygote in *Chorthippus longicornis* which has lost a new small interchanged chromosome (*FE*). The old chromosomes are *AiBC*, *DE*, and *FxG* (Coleman's 3, 7 and 1), the new chromosomes *AiBG*, *FE* and *CxD*. The differential segment is *x*. The corresponding nullisomic, as found in *Godetia*, would be homozygous *AiBG*+*CxD* (after Coleman).

TABLE 12*a*

History of the monosomic

Families 1/40 and 3/40 from sisters in 48/39 crossed reciprocally. The parents are both heterozygous telocentrics with rings-of-four ex-ring-of-ten. The progeny are classified in regard to rings and telocentrics independently.

interchanges \ telocentrics	⊙	⊙	⊙	Total
⊙	–	3	–	3
◔	–	4	1	5
◕	1	2	2	5
◑	–	–	1	1
●	1 (*m*)	–	–	1
Total	2	9	4	15

(*m*) : $1^{12}/40$ monosomic plant with chain of three instead of two pairs, *i.e.* 2(4)+(3)+2(2) = 15

TABLE 12*b*

Sister cross, $3^5/40 \times 1^{12}/40$ *monosomic and reciprocal*

Family	Cross	⊙	◔	◕	◑	●	Total
4/41	♀ ◕ × ♂ ● – 1	4	2	4 *	–	1	11
5/41	Reciprocal	–	10 †	1 ‡	1	–	12

* One was trisomic in some cells for one of the telocentrics, having three telocentrics ($4^{20}/41$).

† One had fragmentation of a second mesocentric chromosome to give two new telocentrics ($5^9/41$).

‡ One had the two telocentrics converted into isochromosomes in some cells ($5^7/41$).

short chromosome has been constructed—an experimental candidate for redundancy of the kind required by our evidence of the evolutionary reduction of chromosome numbers in many families of plants (*cf.* Darlington and Mather, 1949 ; fig. 85).

The progeny of our monosomic, however, show that it is incapable of producing effective $n-1$ gametes (table 12*b*). Its seedling fertility in reciprocal crosses with a sister plant with two rings-of-four was 5 per cent. or half the standard recorded for the two rings-of-four (table 8). The *Campanula* monosomic is therefore, like that in *Chorthippus*, an unsuccessful attempt to do what in *Godetia* has succeeded (*cf.* Hiorth, 1948).

11. NEW TELOCENTRICS AND ISOCHROMOSOMES

New interchanges are demonstrable in other crosses (table 4) but these are not the only new changes to occur.

One trisomic plant ($5^9/41$) of the cross : monosomic by homozygous telocentric with two rings-of-four (table 12*b*) was doubly heterozygous for the telocentric condition (fig. 8A). One of the chromosomes of

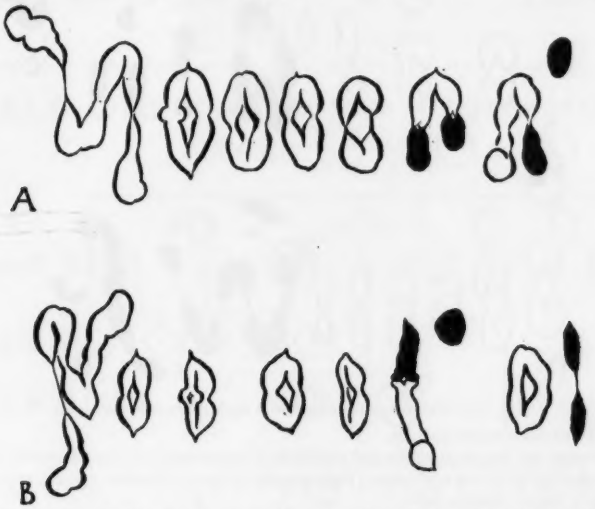


FIG. 8.—Two cells of $5^9/41$ heterozygous for two pairs of telocentrics (in black) one of which is supernumerary, *i.e.* trisomic.

A. One of the trisomic telocentrics is unpaired.

B. One of the disomic telocentrics is unpaired ; two homologous supernumerary telocentrics are paired with one another, *i.e.* they are tetrasomic.

the trisomic association had broken up to give two new telocentrics. This would happen presumably as a result of failure of pairing in the corresponding bivalent at meiosis in either parent followed by complementary misdivision of the two univalents (fig. 12).

The normal homologue of the broken chromosome showed another variation. It was reduplicated in some cells which were therefore trisomic for this chromosome and showed the expected variable configurations, both, one, or neither of the telocentrics pairing with the unbroken chromosome. In addition the telocentrics varied in frequency from cell to cell. Their centromeres, as was to be expected with new telocentrics, were not of the standard activity. In consequence two homologous telocentrics were sometimes found in the same cell and paired (fig. 8B).

As might be foreseen from their erratic behaviour, the new telocentrics seem to have been lost in the bulk of the progeny of

FIG. 9.—Heterozygous telocentric with ring-of-six ($20^1/42$).

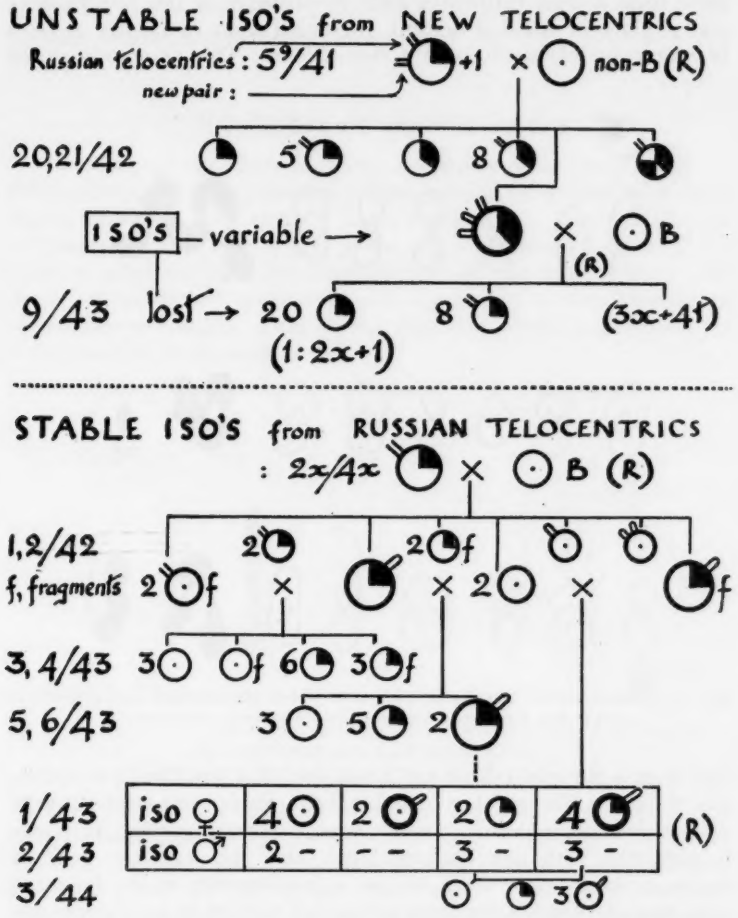
A. Cell without isochromosomes.
B. Cell with two isochromosomes and stickiness of chromosomes ; one telocentric unpaired.
C. Bivalent to which the new minute fragment which arose in families 1 and 2/42 is attached by a triple chiasma ($2^{10}/45$).
D and E. Telocentrics in a chain-of-five ($1^4/42$).
D. With one isochromosome.
E. With two isochromosomes (bivalents omitted).

this plant crossed either way with a non-basic homozygote (20 and 21/42). Out of the 17 seedlings, 15 were heterozygous for the old pair of telocentrics and two had no telocentrics at all (table 13). One of the 15, however ($20^1/42$), had, in addition to the balanced complement, one or two isochromosomes, *i.e.* chromosomes with two identical arms (fig. 9). This unbalance led to stickiness of the

chromosomes at meiosis. The isochromosomes had presumably arisen by *secondary misdivision* (due to sister reunion in the centromere) in

TABLE 13

Family histories of the unstable isochromosomes derived from the Russian telocentrics, crossed with the monosomic to give 59/41 and the stable isochromosomes derived from new telocentrics which arose along with minute fragments and tetraploidy in the 2x/4x chimæra 18/40.



the new and unstable telocentrics. These, as we saw, suffered from the additional disability of being frequently unpaired at meiosis, a circumstance favouring misdivision, primary or secondary.

The isochromosomes varied in number. In some anthers they had been lost and a fixation of the same plant the following year

failed to reveal them. At pre-meiotic mitoses they were evidently liable to undergo further misdivision and, having lagged, they were seen to have been cut by the cell wall.

The progeny of $20^1/42$ included a trisomic and a triploid but no plant with isochromosomes. The isochromosomes in this line may therefore be described as unstable.

In a second line the situation was different (table 13). This line arose from a cross between a basic homozygote of the Xanthi stock and a plant of Russian descent, presumed from its crosses to be a heterozygous telocentric with a ring-of-four. This plant was scored

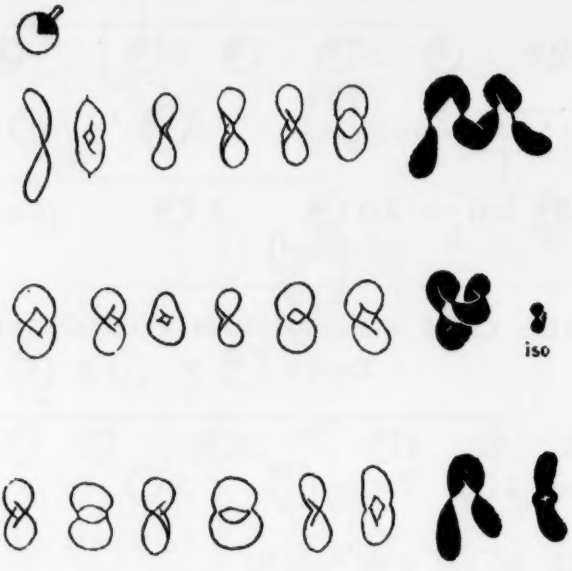


FIG. 10.—Isochromosome homologous with arms of two chromosomes in a ring-of-four and giving, therefore, a ring, or chain-of-five, or its components.

only from a tetraploid shoot and it was doubtless somatically irregular, like $5^9/41$ and like one of its own daughters, for in some crosses it gave no isochromosomes at all. In this cross, however, amongst 12 plants it gave two with one isochromosome and one with two. These isochromosomes were, as in $20^1/42$, supernumerary to the balanced complement. One of the three plants had derived its isochromosome through the pollen, two through the eggs (1 and 2/42). In addition, two of the 12 plants had extra centric fragments which were too small to recognise as telocentrics or isochromosomes. Such fragments may well have arisen from such lagging and cutting of isochromosomes as was seen in $20^1/42$.

One of the isochromosome plants, crossed with a plain sister reciprocally, gave progeny carrying the isochromosome, again both through the pollen and the eggs. Also, two of their sister seedlings, scored as free from isochromosomes or telocentrics (2^5 and $2^8/42$) when intercrossed gave two seedlings with isochromosomes (6^7 and $6^9/43$). One of the parents must therefore have had these chromosomes in a part of its cells. The offspring for the first time showed pairing of the isochromosome with a member of a ring. A number of the various possible configurations were found (fig. 10). But these did not include a ring-of-five.

12. THE ORIGIN OF ISOCHROMOSOMES

In some of the first instances isochromosomes arose early in development to give chimæras. The precise conditions of a later origin, however, have recently come to light in a family 1/48 with 26 plants derived from $9^3/43$ (table 13). Thirteen plants had the usual 7 bivalents and the chain-of-three of the heterozygote. Of these, ten developed isochromosomes, apparently in every anther of several flowers studied. Thus in $1^2/48$ (fig. 11) the following frequencies were found in single anthers :—

1st flower	122 cells	111 normal	11 isochromosomes
2nd „	93 „	85 „	8 „
Total	215 „	196 „	19 „

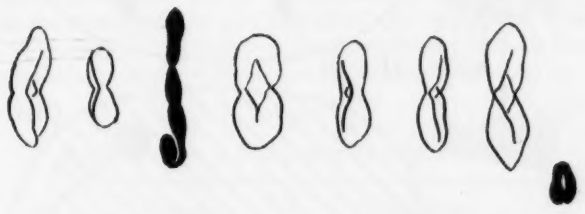


FIG. 11.—Meiosis in the plant $1^2/48$ in which an isochromosome has arisen from one of the two telocentrics at a pre-meiotic division.

In 9 per cent. of cells one telocentric had been transformed into an isochromosome. Since two isochromosomes were never found in one cell, the one was presumably always derived from the same telocentric, one with a weaker centromere. Presumably also, cell conditions were unfavourable for spindle development in some pre-meiotic mitosis. The pre-meiotic stages were therefore examined in preparations of sectioned material.

In the normal development of the anthers of flowering plants the mitoses in the mother-cell precursors are synchronised. In these plants, however, synchronisation was upset (confirming the abnormality

of cell conditions) and study was therefore more difficult. The passage of an undivided telocentric to one pole was, however, seen three times. This process should give (as previously seen in pollen grains) an isochromosome together with a telocentric in one cell and the same telocentric alone in its defective partner cell. Thus, if A. and B. are the two telocentrics and A.B the normal chromosome, mitosis takes the following course (fig. 12) :—

normal $\frac{A.B+A.+B.}{A.B+A.+B.}$ abnormal $\frac{A.B+A.A+B.}{A.B \qquad +B.}$

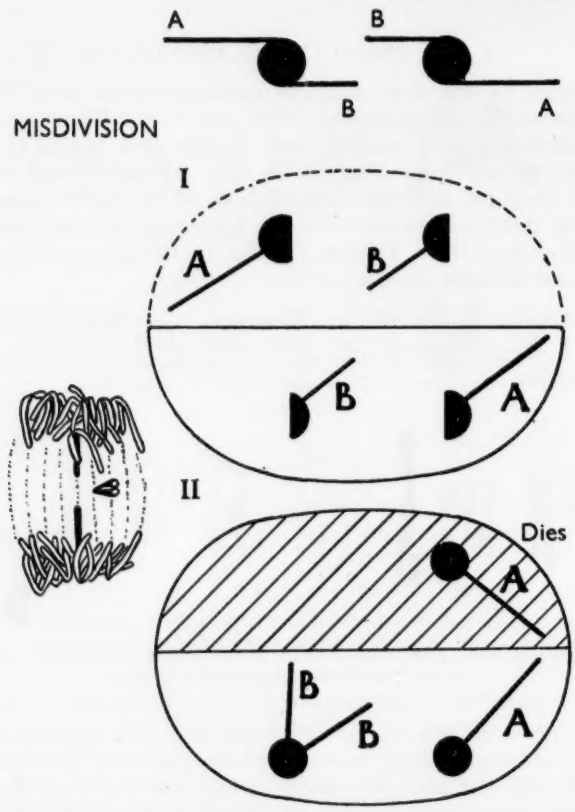


FIG. 12.—Diagram showing the origin of telocentrics by complementary misdivision and the breakdown of one of them by secondary misdivision. Pre-meiotic anaphase showing normal division of one telocentric and secondary misdivision (non-division of centromere) of the other, giving an isochromosome (*cf.* Plate I, fig 6). ×3200.

It is therefore remarkable that the corresponding 9 per cent. of defective pollen mother-cells were not found. The secondary mis-

division of the centromere and the origin of the isochromosomes take place two or three mitoses before meiosis and in the intervening period the complementary defective cells must degenerate and disappear. This process of complementary origin and elimination seems to account for the apparent simple transformation of telocentrics into isochromosomes in so many of our plants.

At the same time it will be seen that the systematic elimination of a defective type of cell regularly arising is a notable example of the principle that natural selection must operate rigorously at the cell level if plants and animals are to attain to a uniform and healthy maturity.

In the abnormal plants studied in 1948 (although not in 1949) there was a failure of correct differentiation of the anther and other flower parts which were malformed and sometimes doubled. This error of form was no doubt the external sign of an internal upset in development of which the non-synchronisation of mitoses and the genetic change in the chromosomes were internal symptoms. The regularity in the irregularity enabled us to trace the causal sequence of what might otherwise have been an elusive and conjectural property.

13. POLLEN-GRAIN INHERITANCE

A Feulgen technique was developed for studying pollen-grain mitoses in 1946. The normal pollen grains in the diploid *Campanula* have 8 chromosomes with nearly but not quite equal arms. One of them has a wide centric constriction: the nucleolar organiser adjoins the centromere to give this effect (see appendix). The pollen grains of the plant $1^2/48$ with one unstable telocentric had a distribution of the eighth autosome A.B, or A.+B., as shown in table 14.

TABLE 14

Distribution of the A.B autosome in 74 pollen grains of $1^2/48$

Balanced		Unbalanced	
+A.B.	38	+A.A	6
		+A.B.+A.A	3
		+A.A.+B	1
+A.+B.	22	+A.B +. B. (or A.)	2
		+B. (or A.)	2
Totals	60		14

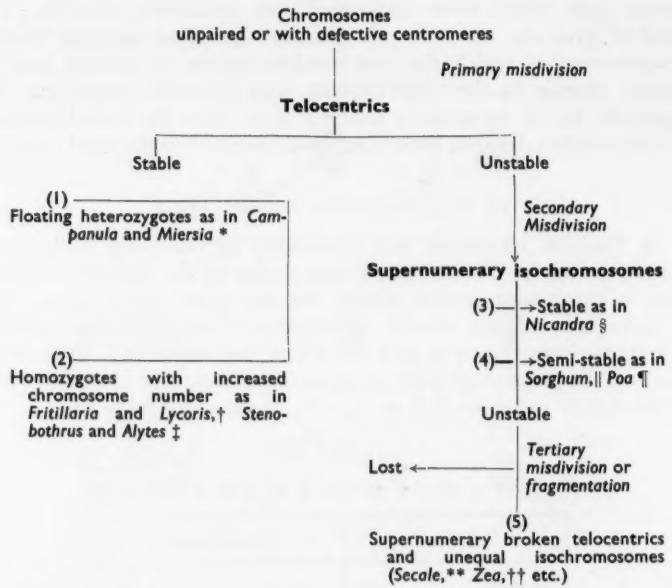
Thus 19 per cent. of the pollen grains were unbalanced and most of them had the isochromosome A.A. This indicates a still higher frequency of isochromosomes in the mother-cells or, *i.e.* more

than that in the mother-cell samples examined. A further point of interest is that 8 of the 74 pollen grains are defective in respect of an A. or B. arm : they are nullisomic for these half-chromosomes. Thus, although such cells are eliminated before meiosis they are not eliminated in the young pollen grain.

14. THE FUTURE OF ISOCHROMOSOMES

Apart from the two isochromosome lines we have the 236 seedlings of plants with stable and regularly paired telocentrics, heterozygous or homozygous. In all these no plant occurred with an isochromosome. Doubtless in these plants failure of pairing would have led to the

TABLE 15



* Cave and Bradley, 1943
† Darlington and Janaki Ammal, 1945*a*
‡ Wickbom, 1949
§ Darlington and Janaki Ammal, 1945*b*
‖ Darlington and Thomas, 1941
¶ Müntzing, 1948
** Müntzing, 1944
†† Darlington and Upcott, 1941

origin of isochromosomes from the telocentrics at meiosis. In its absence, however, isochromosomes can arise even at mitosis but only, as we see, where the centromere of the telocentric is untested by natural selection and unstable. For this reason they appear as supernumeraries to the ordinary complement.

Like new telocentrics isochromosomes may turn out to be stable, having a competent centromere. If unstable they will be irregularly

distributed even at mitosis and liable to further misdivision and fragmentation. But if stable they may be transmitted by pollen and eggs and become established in the species as in *Nicandra* (Darlington and Janaki Ammal, 1945*b*). Such stable isochromosomes are probably very common, *e.g.* in *Haworthia Browneana* (Resende and Franca, 1946) and *Tulipa saxatilis*, 3*x* (Upcott and Philp, 1939). And again both telocentrics and isochromosomes may be stable in some tissues, and not in others, giving the condition for differentiation as in *Sorghum*. From such chromosomes probably arise the bulk of supernumerary fragments which, as in maize and rye, betray their origin by an inadequacy of the centromere at one stage or another. Thus we may represent the historical alternatives diagrammatically as in table 15.

15. THE EVOLUTION OF CHROMOSOME NUMBERS

Telocentric chromosomes seem to arise in *Campanula* as elsewhere by misdivision following failure of pairing at meiosis. These conditions occur in all sexual organisms, although some species and races, like our Russian stocks, are, it seems, genotypically predisposed to suffer misdivision more frequently than others. Three new origins of telocentrics in our experiments all occurred in descendants of the Russian stocks. Survival, however, will depend on other circumstances. Two complementary telocentrics must arise, as we saw, by double misdivision at the same time to give the simple " fragmentation " of a chromosome so often imagined to account for change of number without change of balance. Single misdivision will permit only of supernumerary fragments such as are widely known and such as we found in certain families. There is then the mechanical obstacle to be overcome. If the centromere is split so as to give too small a fraction to one of the telocentrics it will presumably be lost, as ours was in families 20 and 21/42, unless it forms an isochromosome with twice as large a centromere. The possibilities of misdivision of the centromere will vary as between species but we must expect that in most of them only a lucky few of the new telocentrics will survive.

These conditions, difficult though they are, are no doubt fulfilled often enough to make the origin of new telocentrics an important means of increasing the basic number of chromosomes, and with it the number of linkage groups and even the balance of the nucleus as well. The differences in stability of basic numbers of chromosomes in different groups are well known and are strikingly shown by a survey of chromosome numbers in plants (Darlington and Janaki Ammal, 1945*a*). It would now seem that they depend to some extent on differences in the structure and stability of the centromere in the different groups.

At the same time our monosomic reveals the means of achieving the more difficult end of reducing the basic number of chromosomes.

Its association with interchange shows the common principle at work in all such cases.

Instability of chromosome number has the common advantages and disadvantages of setting up a genetic isolation of old and new forms. In our present experiments we have used it as an additional hybridity marker. We have marker chromosomes instead of marker genes. The telocentrics, like the interchanges, show the constant action of selection in favouring the hybridity equilibrium which has been established as normal by the breeding habit of the stock concerned.

16. CONCLUSIONS

1. Incipient complex heterozygotes with ring-formation have been produced artificially by crossing in *Campanula persicifolia*.

2. They show different degrees of true-breeding in regard to ring-formation according to the degree of inbreeding that is practised: the greater the inbreeding, the greater the selection for hybridity as revealed by structural hybridity. This confirms the evolutionary trend inferred from the comparison of breeding behaviour and ring-formation in *Oenothera*.

3. The rings break down by crossing-over, which gives half-mutants with lower rings. The higher rings also give about 10 per cent. of trisomics whose extra chromosomes are largely eliminated in breeding even on the female side.

4. Selection for ring-size compels us to disregard selection for vigour and fertility. Vigour is steadily reduced with increasing size of the ring. The annual habit is favoured at the expense of the biennial. Fertility is also reduced in higher rings by the less regular segregation.

5. Apart from the five interchanges combined in the ring-of-twelve, four new ones have arisen in the experiments, and four other new ones have been found in natural populations, three of them in Russia.

6. In a Russian population one plant had a chromosome split by misdivision of the centromere into two telocentrics. Homozygous derivatives were bred with 9 bivalents instead of 8. Combined with the rings in heterozygous condition they gave chains of 7, 11, 15, etc. New telocentrics arose three times.

7. Both old and new telocentrics in some genetic combinations gave isochromosomes by secondary misdivision either early or late in development. The isochromosomes were sometimes unstable, sometimes stable, being carried as supernumeraries by pollen and eggs.

8. Cells deficient for chromosome arms were formed as a result of segregation or secondary misdivision. They were lethal to pollen mother-cell precursors but not to young pollen grains.

9. A monosomic plant arose deficient for a new interchanged chromosome which had probably been produced at the parental meiosis. This shows how the basic number of chromosomes can be reduced just as the telocentrics show how it can be increased.

17. SUMMARY

Experiments with ring-formation in *Campanula persicifolia* and the origin of interchanges, monosomics, telocentrics and isochromosomes reveal the principles by which the chromosomes are organised and their basic numbers changed up and down. Structurally hybrid types are used as a means of identifying the opposed processes of selection at work with opposed habits of inbreeding and outbreeding. They confirm our knowledge of the conservative nature of the breeding system. High rings are not genetically advantageous enough for survival in an outbreeding species. Their development is favoured by a transition to inbreeding such as is assumed to have occurred in *Oenothera*.

APPENDIX

COMPOUND CONSTRICTIONS

L. F. LA COUR

We find in *Campanula persicifolia* a chromosome pair differing from the remainder of the complement at mitosis in the root tip and in the pollen grain. They have extremely wide centric constrictions. As a rule, indeed, the arms are so wide apart as to appear completely separated. They further diverge in the behaviour of their arms. At metaphase, the chromatids in one arm are widely separated and, as a rule, lie flat in the plate. In the other arm, the chromatids are usually found close together and lying in an axial plane. In other words, the arms are relatively rotated through a right-angle on opposite sides of the centromere (figs. 13, 14 ; plate II, fig. 4).

We first supposed that these chromosomes might be associated with the origin of telocentrics in certain strains. This, however, is not the case for they are present equally in plants with telocentrics and isochromosomes. Moreover, their behaviour at anaphase is strictly normal ; misdivision of their centromeres has not been seen and neither do they separate before the other chromosomes.

The singular behaviour of the arms would seem to be due to an eccentric position of the centromere. Presumably it is nearer the arm in which the chromatids remain together. This, together with the wide separation of the arms, implies that the unspiralised thread is lengthened in some way on one side of the centromere.

Two possibilities come to mind as to providing an explanation for this condition : (*a*) unspiralised heterochromatin at one side of the centromere or (*b*) a close proximity of the nucleolar organiser.

Heterochromatin is not perceptible in the resting nuclei in this species and we cannot therefore reach any conclusion as to its position on

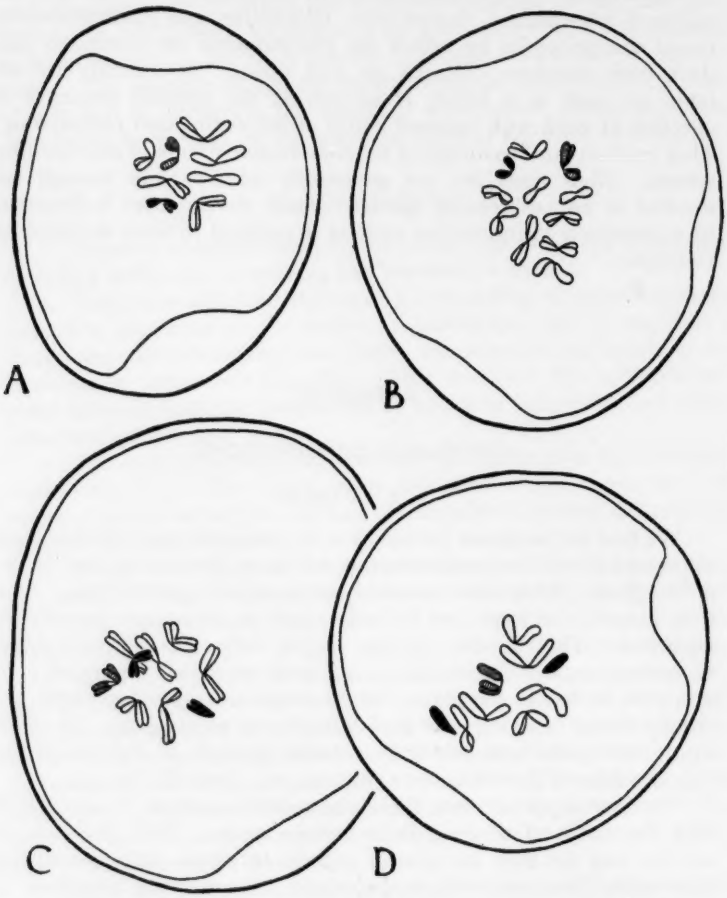


FIG. 13.—Four pollen-grain mitoses, showing the nucleolar chromosome (with wide centric constriction) stippled, and telocentrics and isochromosomes black.

A. Defective nucleus $7+i$ and B. $10+i$, both from $2^{8}/43$.

C. Isochromosome newly arisen from telocentric in the unstable plant $1^{2}/48$ of which D. is a normal pollen grain. × 1200.

the chromosomes. The pollen-grain mitosis, however, where the prophases are very favourable for study, provides the answer to the problem.

At metaphase a single chromosome shows the wide centric constriction and singular behaviour in the chromatids. The prophases

FIG. 14.—Root-tip mitoses, showing the wide constriction due to the combined centromere and nucleolar organiser (*n*).
A. Unstable plant $1^{1}/48$ with two telocentrics (*t*).
B. Plant $2^{5}/43$ with an isochromosome (*i*). ×3400.

show this chromosome attached to the nucleolus, the two arms lying freely extended : the position of the organiser obviously adjoins the centromere (fig. 15).

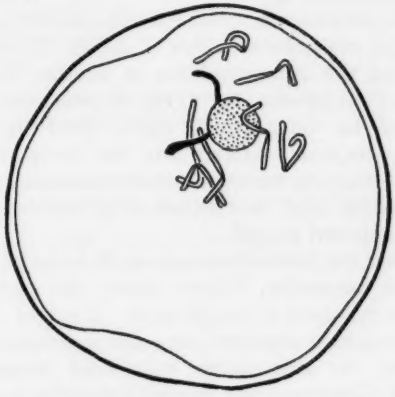


FIG. 15.—Late prophase of pollen-grain mitosis in a basic plant showing the median attachment of one chromosome to the nucleolus, the cause of the wide, compound, constriction. ×1200.

A remarkably similar situation seems to occur in *Poa alpina*. Müntzing (1948) in this species, has observed a chromosome pair with accentuated centric constrictions and he concludes that they are the nucleolar chromosomes. He describes them as having a short arm, more intensely staining and broader than the long arm, and he infers that this arm is wholly heterochromatic. The apparent dissimilarity in the broadness of the arms may, however, be due, as in *Campanula*, to a difference in rotation of the arms on the two sides of the centromere. The distinction is perhaps less noticeable

in *Poa*, for it is only in one of Müntzing's four illustrations (fig. 25) that the chromatids appear separated.

In *Pisum* we find what appears to be a more direct association of centromeres and nucleoli both in pollen and in root-tip mitoses. It is a unique situation in which all the chromosomes take part, each centromere region giving rise to a nucleolus at telophase (Håkansson and Levan, 1942). This behaviour, however (which I have confirmed), does not lead to any apparent widening of the centric constriction. We must assume therefore, that in this case, the nucleoli are organised direct from the centromeres.

There are of course other examples where, for reasons unknown, the centric constrictions appear accentuated, *e.g.* in *Aconitum* (Darlington, 1937) and in some species of *Crocus* (Mather, 1932). In *Aconitum* it is apparent in all the chromosomes of the complement while in *Crocus* it is usually most obvious in chromosomes associated with nucleoli formed close to the centromere. In these cases, however, we see a secondary constriction and we cannot suppose that it leads to an accentuation of the primary constriction. It may be, of course, that in these and some other cases, the wide constrictions are due to heterochromatin.

In *Fritillaria lusitanica* heterochromatin occurs on both sides of the centromere in most chromosomes of the set (Botelho and Mendes, 1946) and I find the same condition in another Iberian species *F. pyrenaica*. Like *Paris japonica*, but unlike *F. pudica* and *Trillium* species (Darlington and La Cour, 1940, 1941), these show their heterochromatin at metaphase without any low temperature treatment. A cleaner cut distinction between heterochromatin and euchromatin, in regard to nucleic acid concentration, is, however, obtained when the plants are subjected to cold.

It may be that the heterochromatin in *F. lusitanica* and *F. pyrenaica*, because of close proximity, follows closely the cyclic behaviour of the centromeres in regard to nucleic acid. It might then be rendered visible, as understained segments, at normal temperatures. In these species, however, the arms within individual chromosomes are not wide apart as in *Campanula*, the starved heterochromatic constrictions are of an even thickness, approximately two-thirds the diameter of the two chromatids. It may be that in *Aconitum* and *Crocus* an unspiralised condition of the heterochromatin, as in *Paris japonica*, gives a picture akin to that of *Campanula*.

18. REFERENCES

BOTELHO, M., AND MENDES, E. J. 1946.
Caryological studies on *Fritillaria lusitanica*.
Portug. Acta Biol., *1*, 310.

CATCHESIDE, D. G. 1936.
Origin, nature and breeding behaviour of *Oenothera Lamarckiana* trisomics.
J. Genet., *33*, 1-23.

CAVE, M. S., AND BRADLEY, M. V. 1943.
Alteration of chromosome number in *Miersia chilensis*.
Amer. J. Bot., *30*, 142-149.

CLELAND, R. E. 1944.
The problem of species in *Oenothera*.
Amer. Nat., *78*, 5-28.

COLEMAN, L. C. 1947.
Chromosome abnormalities in an individual of *Chorthippus Longicornis* (Acrididæ).
Genetics, *32*, 435-447.

DARLINGTON, C. D. 1931.
The cytological theory of inheritance in *Oenothera*.
J. Genet., *24*, 405-474.

DARLINGTON, C. D. 1932, 1937.
Recent Advances in Cytology. London : Churchill.

DARLINGTON, C. D. 1939.
The Evolution of Genetic Systems. Cambridge : University Press.

DARLINGTON, C. D. 1940*a*.
The origin of isochromosomes.
J. Genet., *39*, 351-361.

DARLINGTON, C. D. 1940*b*.
The prime variables of meiosis.
Biol. Rev., *15*, 307-322.

DARLINGTON, C. D., AND GAIRDNER, A. E. 1937.
The variation system in *Campanula persicifolia*.
J. Genet., *35*, 97-138.

DARLINGTON, C. D., AND JANAKI AMMAL, E. K. 1945*a*.
Chromosome Atlas of Cultivated Plants. London : Allen & Unwin.

DARLINGTON, C. D., AND JANAKI AMMAL, E. K. 1945*b*.
Adaptive isochromosomes in *Nicandra*.
Ann. Bot., *9*, 267-281.

DARLINGTON, C. D., AND LA COUR, L. F. 1940.
Nucleic acid starvation of chromosomes in *Trillium*.
J. Genet., *40*, 185-213.

DARLINGTON, C. D., AND LA COUR, L. F. 1941.
The detection of inert genes.
J. Hered., *32*, 115-121.

DARLINGTON, C. D., AND THOMAS, P. T. 1941.
Morbid mitosis and the activity of inert chromosomes in *Sorghum*.
P.R.S., B, *130*, 127-150.

DARLINGTON, C. D., AND UPCOTT, M. B. 1941.
The activity of inert chromosomes in *Zea Mays*.
J. Genet., *41*, 275-296.

GAIRDNER, A. E. 1937.
The segregation of six factors in *Campanula persicifolia*.
J. Genet., *35*, 281-288.

GAIRDNER, A. E., AND DARLINGTON, C. D. 1931.
Ring-formation in diploid and polyploid *Campanula persicifolia*.
Genetica, *13*, 113-150.

HÅKANSSON, A. 1945.
Zytologische studien an monosomischen typen von *Godetia Whitneyi*.
Hereditas, *31*, 129-162.

HÅKANSSON, A. 1946.
Meiosis in hybrid nullisomics and certain other forms of *Godetia Whitneyi*.
Hereditas, *32*, 498-513.

HÅKANSSON, A., AND LEVAN, A. 1942.
Nucleolar conditions in *Pisum*.
Hereditas, *28*, 436-440.

HIORTH, G. 1948.
Zur genetik der monosomen von *Godetia Whitneyi*.
Z.I.A.V., *82*, 230-275.

MATHER, K. 1942.
The balance of polygenic combinations.
J. Genet., *43*, 309-336.

MÜNTZING, A. 1944.
Cytological studies of extra fragment chromosomes in rye. I. Iso-fragments produced by misdivision.
Hereditas, *30*, 231-248.

MÜNTZING, A. 1948.
Accessory chromosomes in *Poa alpina*.
Heredity, *2*, 49-61.

RESENDE, F., AND DA FRANCA, P. 1946.
Sur l'origine de nouvelles formes.
Portug. Acta Biol., *1*, 289-307.

STADLER, L. J. 1931.
The experimental modification of heredity in crop plants I.
Sci. Agric., *11*, 557-572.

STOMPS, T. J. 1931.
Weiteres über parthenogenesis bei *Oenothera*.
Ber. dtsch. bot. Ges., *49*, 258-266.

UPCOTT, M., AND PHILP, J. 1939.
The genetic structure of *Tulipa*. IV. Balance, selection and fertility.
J. Genet., *38*, 91-123.

WICKBOM, T. 1949.
A new list of chromosome numbers in *Anura*.
Hereditas, *35*, 242-245.

PLATE I.—Smears of anthers of *Campanula persicifolia*.

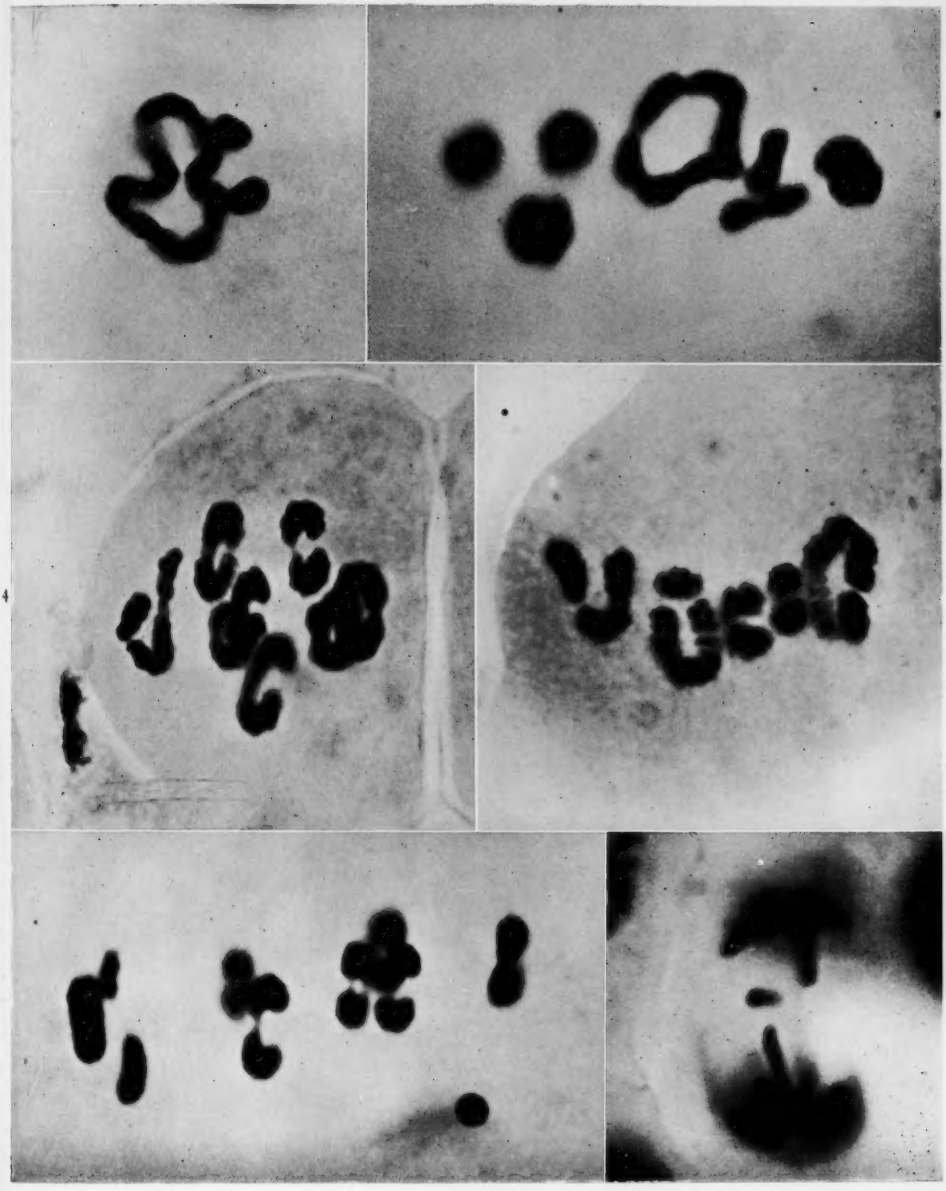


FIG. 1.—Ring-of-twelve and two pairs, (12)+2(2), at meiosis in $6^{8}/38$. ×3000.
FIG. 2.—Homozygous telocentric and heterozygous interchange plant $1^{14}/40$, with ring-of-six and 6 bivalents : $2n = 18$. *Cf.* text fig. 5. ×3000.
FIG. 3.—Monosomic plant 2(4)+(3)+2(2), $1^{12}/40$, $2n = 15$. ×2000
FIGS. 4, 5, 6.—Heterozygous telocentric plant $2^{1}/48$.
FIG. 4.—Chain-of-three in normal pollen mother-cell. ×2000.
FIG. 5.—One of the telocentrics has been converted into an isochromosome which is unpaired having formed a chiasma with itself and is lying off the plate. The remaining telocentric is paired with its large partner. ×3000.
FIG. 6.—Origin of the isochromosome from a lagging telocentric in a premeiotic mitosis. ×3000.

PLATE II.—Pollen-grain mitoses in diploid plants with telocentrics and isochromosomes. ×2400.

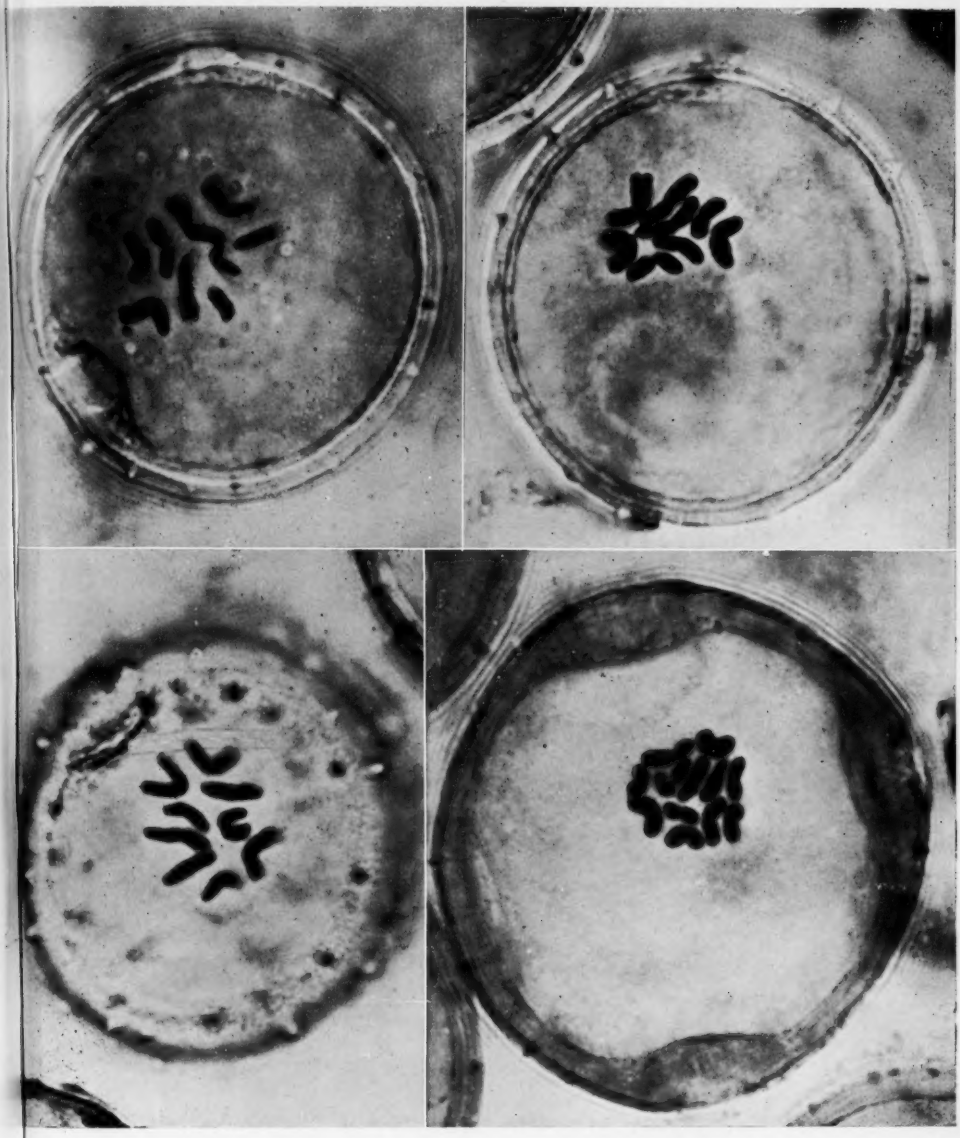


FIG. 1.—Seven autosomes and 2 telocentrics at 2 and 4 o'clock; note that one has its centric end pointing outward indicating a weak centromere (*cf.* text fig. 12).
FIG. 2.—Defective nucleus: 7 autosomes and 1 isochromosome (2 o'clock).
FIG. 3.—Eight autosomes and 1 isochromosome (3 o'clock).
FIG. 4.—Eleven autosomes and 1 isochromosome (10 o'clock); note the chromosome with the compound constriction has widely separated chromatids in the shorter arm with the nucleolar organiser and therefore seems tripartite. The three extra autosomes are due to irregular segregation in a ring.

BOOKS AND SPECIAL VOLUMES IN THE FIELD OF GENETICS, 1939-1949

ISOLDE BEHR

John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.

Received 1.x.49

AKERMAN, A. *et al.* (ed.). 1948. *Svalöf, 1886-1946. History and Present Problems.* Pp. 389. Lund : Karl Bloms Boktrykeri.

ALTENBURG, E. 1945. *Genetics.* Pp. xii+452. New York : Henry Holt & Co.

ANDERSEN, P. F. 1942. *Inheritance of Harelip and Cleft Palate.* Copenhagen : E. Munksgaard.

ANDERSON, E. 1949. *Introgressive Hybridisation.* Pp. ix+109. New York and London : Chapman & Hall (New York : John Wiley).

ANDREASSEN, M. 1943. *Hæmofili i Danmark.* Pp. 168. Copenhagen : E. Munksgaard.

ASHTON, T. 1946. *The Use of Heterosis in the Production of Agricultural and Horticultural Crops.* Pp. 34. Cambridge : Imperial Bureau of Plant Genetics.

BAKER, J. R. 1945 (1933). *Cytological Technique.* 2nd ed. Pp. vii+211. London : Methuen.

BARLOW, N. (ed.). 1945. *Charles Darwin and the Voyage of the Beagle.* Pp. 279. London : Pilot Press.

BARTELS, E. D. 1941. *Heredity in Graves Disease.* Pp. 384. Copenhagen : E. Munksgaard.

BARTELS, E. D., AND BRUN, G. 1943. *Gipsies in Denmark.* Pp. 179. Copenhagen : E. Munksgaard.

BARTON, L. V., AND CROCKER, W. 1948. *Twenty Years of Seed Research at the Boyce Thompson Institute for Plant Research.* Pp. 148. London : Faber & Faber.

BAUDIN, L. 1946. *Introduction à la Biologie.* Pp. 224. Lausanne : Payot.

BAUER, J. 1945 (1942). *Constitution and Disease.* 2nd ed. Pp. ix+208. New York : Gruene & Stratton.

BELL, A. G. 1948. *Inheritance of Deafness.* Washington : Volta Publ.

BENEDICT, R. 1942. *Race and Racism.* Pp. viii+175. London : Routledge & Sons.

BIBBY, C. H. 1939. *Heredity, Eugenics and Social Progress.* Pp. 93. London : Gollancz, New People's Library.

BIBBY, C. H. 1939. *Evolution of Man and his Culture.* Pp. 92. London : Gollancz, New People's Library.

BLACKER, C. P. 1947. *Eugenics in Prospect and Retrospect* (Galton Lecture 1945). Pp. 33. London : H. Hamilton.

BOND, A. DE M. 1940. *An Experiment in the Teaching of Genetics.* Pp. viii+99. New York : Columbia University Press.

BONNIER, G., AND TEDIN, O. 1940. *Biologisk Variationsanalysis* (*Analysis of Variance in Biology*). Pp. 325. Stockholm.

BOUNOURE, L. 1939. *L'Origine des cellules reproductices et le problème de la ligne germinale.* Pp. xii+272. Paris : Libr. Gauthier-Villars.

BOUNOURE, L. 1940. *Continuité germinale et reproduction agame.* Pp. iv+84. Paris : Libr. Gauthier-Villars.

BOURNE, G. (ed.). 1942. *Cytology and Cell Physiology.* Pp. xii+296. Oxford : Clarendon Press.

BOYD, W. C. 1939. *Blood Groups* (Reprint from *Tabulæ Biologicæ, 17*). Pp. 128. Den Haag : W. Junk.

BRIDGES, C. B., AND BREHME, K. S. (ed.). 1944. *The Mutants of* Drosophila melanogaster. Pp. vii+257. Washington : Carnegie Institution.

BURCH, G. I., AND PENDELL, E. 1947. *Human Breeding and Survival.* New York : Pelican Books.

BURGEFF, H. 1943. *Genetische Studien an Marchantia. Einführung einer neuen Pflanzenfamilie in die genetische Wissenschaft.* Pp. 296. Jena : Gustav Fischer Verlag.

BURLINGAME, L. L. 1940. *Heredity and Social Problems.* Pp. xi+369. New York : McGraw Hill.

CAIN, S. E. 1944. *Foundations of Plant Geography.* Pp. xiv+556. New York : Harper & Brothers.

CAMBRIDGE UNIVERSITY PRESS. 1939-49. *Treasury of Human Inheritance* (various editions). Cambridge.

CARLES, J. 1945. *Problèmes d'Hérédité.* Pp. 258. Paris : Beauchesne et ses Fils.

CASTLE, W. 1940. *Mammalian Genetics.* Pp. viii+169. Cambridge, Mass. : Harvard University Press.

CASTLE, W. 1940. *Manual for a Laboratory Course on Genetics.* Pp. v+33. Cambridge, Mass. : Harvard University Press.

CAULLÉRY, M. 1942. *L'Organisme et Sexualité.* Paris.

CHANDRASEKHARAN, S. N., AND PARTHASARATHY, S. V. 1948. *Cytogenetics and Plant Breeding.* Pp. xvi+589. Madras : P. Varadachary & Co.

CLARK, H. W. 1940. *Genes and Genesis.* Pp. v+155. Mountain View, Cal. : Pacific Publications.

CLAUSEN, J. C. 1945. *Experimental Studies in the Nature of Species.* Vol. 2. Plant evolution through amphiploidy and autoploidy, with examples from the *Madiæ.* Pp. vii+174. Washington : Carnegie Institution.

COLD SPRING HARBOR. *Symposia on Quantitative Biology.* New York.
1941. Vol. 9. Genes and chromosome structure and organisation. Pp. x+316.
1946. Vol. 11. Heredity and variation in micro-organisms. Pp. xi+314.
1948. Vol. 13. Biological applications of tracer elements. Pp. xi+222.

COLIN, E. 1947 (1941). *Elements of Genetics.* Pp. xiii+402. Philadelphia : Blakiston Co.

COOK, R. C., AND BURKS, B. S. 1946. *How Heredity Builds Our Lives.* Pp. 64. Washington : American Genetical Association.

COTT, H. B. 1940. *Adaptive Coloration in Animals.* (Introduced by J. Huxley.) Pp. xxxii+508. London : Methuen.

CRABB, A. R. 1947. *The Hybrid Cornmakers. Prophets of Plenty.* Pp. xxv+331. New Brunswick : Rutgers University Press.

CRANE, M. B., AND LAWRENCE, W. J. C. 1947 (1934, 1938). *The Genetics of Garden Plants.* Pp. xvii+299. London : MacMillan & Co.

CREW, F. A. E. 1947. *Genetics in Relation to Clinical Medicine.* Pp. 123. Edinburgh : Oliver & Boyd.

CROCKER, W. 1948. *Growth of Plants. Twenty Years Research at the Boyce Thompson Institute.* Pp. v+459. New York : Reinhold Publ. Co.

CUMMINS, H., AND MIDLO, C. 1943. *Finger Prints, Palms and Soles. An Introduction to Dermatoglyphics.* Pp. 309. Philadelphia.

DAHLBERG, G. 1940. *Arv och Ras* (*Heredity and Race*). Pp. 195. Stockholm.

DAHLBERG, G. 1940. *Statistical Methods for Medical and Biological Students.* Pp. ix+232. London : Allen & Unwin.

DAHLBERG, G. (translated by L. Hogben). 1942. *Race, Reason and Rubbish.* Pp. 240. London : Allen & Unwin.

DAHLBERG, G. 1947. *Mathematics of the Genetics of Populations.* Pp. viii+138. Basle : Karger.

DAHLBERG, G. 1947. *Mathematical Methods for Population Genetics.* Pp. viii+183. New York : Interscience. Basle : Karger.

DANGEARD, P. 1947. *Cytologie Végétale et Cytologie Générale.* Pp. 616. Paris : Paul Lechevalier.

DARLINGTON, C. D. 1947 (1939). *The Evolution of Genetic Systems.* Pp. xi+149. Cambridge : Cambridge University Press.

DARLINGTON, C. D. 1948. *La Evolucion de los Sistemas Geneticos.* (Translated by F. A. Saez of *The Evolution of Genetic Systems.*) Pp. 197. Buenos Aires : Espasa Calpe Argentina.

DARLINGTON, C. D., AND JANAKI AMMAL, E. K. 1945. *Chromosome Atlas of Cultivated Plants.* Pp. 397. London : Allen & Unwin.

DARLINGTON, C. D., AND LA COUR, L. F. 1947 (1942). *The Handling of Chromosomes.* 2nd ed. Pp. 165. London : Allen & Unwin.

DARLINGTON, C. D., AND MATHER, K. 1949. *Elements of Genetics.* Pp. 446. London : Allen & Unwin.

DARLINGTON, C. D. AND MATHER, K. 1949. *Genes, Plants and People : Essays on Genetics.* Pp. 192. London : Allen & Unwin.

DARWIN, C. 1949. *The Origin of Species.* Reprint of 1st ed. with introduction by C. D. Darlington. London : Watts & Co.

DAVENPORT, C. B. *et al.* 1940. *Medical Genetics and Eugenics.* Pp. 141. Philadelphia : Women's Medical College, Pa.

DEMEREC, M. *et al.* 1941. *Cytology, Genetics and Evolution.* Pp. 168. Philadelphia : University of Pennsylvania Press.

DEMEREC, M. (ed.). 1947 to date. *Advances in Genetics.* 1947. Vol. 1. Pp. xvi+458. 1948. Vol. 2. Pp. viii+373. New York : Academic Press.

DOBZHANSKY, TH. 1937, 1941. *Genetics and the Origin of Species.* Pp. xviii+446. New York : Columbia University Press.

DOBZHANSKY, TH. 1944. *Contributions to the Genetics, Taxonomy and Ecology of* Drosophila pseudo-obscura *and Its Relatives.* Pp. iii+183. New York : Carnegie Institution.

DUNN, L. C., AND DOBZHANSKY, TH. 1946. *Heredity, Race and Society.* New York : Pelican Books.

ERIKSEN, K. 1943. *Heredity Forms and Sterility in Cattle.* I. Biological and genetical investigations. Pp. 155. Lund : University of Lund.

ERNST, A. 1942. *Vererbung durch labile Gene.* Pp. 567. Zürich : Orell-Füssli (Archiv d. Jul. Klaus Stift.).

FISCHER, A. 1946. *Biology of Tissue Cells. Essays.* Pp. viii+348. London.

FISHER, R. A. 1930, 1940. *The Genetical Theory of Natural Selection.* Pp. xiv+272. Oxford : Clarendon Press.

FISHER, R. A. 1925-47. *Statistical Methods for Research Workers.* Pp. xiv+354. Edinburgh : Oliver & Boyd.

FISHER, R. A. 1935-47. *The Design of Experiments.* Pp. xi+260. Edinburgh : Oliver & Boyd.

FISHER, R. A. 1949. *The Theory of Inbreeding.* Pp. viii+120. Edinburgh : Oliver & Boyd.

FISHER, R. A., AND YATES, F. 1938-48. *Statistical Tables for Biological, Agricultural and Medical Research.* Pp. viii+112. Edinburgh : Oliver & Boyd.

FITT, A. B. 1941. *Seasonal Influence on Growth, Function and Inheritance.* Pp. xi+182. Oxford : New Zealand Council Publication.

FLORKIN, M. 1940. *Biochemical Evolution.* Pp. 210. New York.
(1944. *L'Evolution Biochimique.* ed. Desoer. Pp. 212. Liege.)

FOGH-ANDERSON, P. 1942. *Inheritance of Harelip and Cleft Palate.* Pp. 226. Copenhagen : A. Busck, Nyt Nordisk Forlag.

FORD, E. B. 1931-45. *Mendelism and Evolution.* Pp. xii+122. London : Methuen.

FORD, E. B. 1942. *Genetics for Medical Students.* Pp. 162. London : Methuen.

FRANCIS, T. 1944. *The Development of the Pituitary at Hereditary Anterior Pituitary Dwarfism in Mice.* Pp. 154. Copenhagen : E Munksgaard.

FRASER-ROBERTS, J. A. 1940. *An Introduction to Medical Genetics.* Pp. xxiii+266. London : Oxford University Press.

GAGNEBIN, E. 1943. *Le Transformisme et l'Origine de l'Homme.* Pp. 173. Lausanne : Rouge et Cie.

GANDER, M. n.d. (*circa* 1946). *Die Abstammungslehre.* Pp. 180. Einsiedeln : Benziger & Cie.

GARNER, R. J. 1947. *Grafter's Handbook.* Pp. 223. London : Faber & Faber.

GATES, R. R. 1946. *Human Genetics.* 2 vols. Vol. 1, pp. xvi+742 ; Vol. 2, pp. 743-1518. New York : MacMillan & Co.

GATES, R. R. 1949. *Pedigree of Negro Families.* Pp. vii+267. Philadelphia : Blakiston Co.

GATES, R. R. 1948. *Human Ancestry from a Genetical Point of View.* Pp. 438. Cambridge, U.S.A. : Harvard University Press.

GEITLER, L. 1942. *Schnellmethoden der Kern-Chromosomen-Untersuchung.* 2nd rev. ed. Pp. 28. Berlin : Bornträger.

GLASS, B. 1943. *Genes and the Man.* Pp. xii+386. New York : Teachers' College, Columbia University.

GOLDSCHMIDT, R. 1940. *Physiological Genetics.* Pp. 375. New York and London : McGraw Hill.

GOLDSCHMIDT, R. 1940. *The Material Basis of Evolution.* Pp. 448. New Haven and London : Oxford University Press. Yale University Press.

GOLDSTEIN, P. 1947. *Genetics is Easy.* Pp. v+72. Hoboken : Garlan Publications (Handbooks for Teachers and Pupils).

GRAEBNER, T. C. 1948. *Evolution, an Investigation and a Criticism.* Pp. 148. Milwaukee : North-Western Publication House.

GRESSON, R. A. R., AND CLARK, H. HESLOP. 1948. *Essentials of General Cytology.* Pp. viii+184. Edinburgh : University Press.

GRÜNEBERG, H. 1947. *Animal Genetics and Medicine.* Pp. xii+296. London : Hamish Hamilton.

GUILLIERMOND, A. 1941. *The Cytoplasm of the Plant Cell* (translated from MSS. by L. R. Atkinson.) Pp. 257. Waltham : Chronica Botanica.

GUPTA, N. N. S. 1941. *Heredity in Mental Traits.* Pp. xii+207. London : MacMillan & Co.

GUYÉNOT, E. 1941. *L'Idée d'Evolution.* Pp. xxi+462. Paris : A. Michel (Les Sciences de la Vie aux 17 and 18 Siècles).

GUYÉNOT, E. 1944. *L'Origine des Espèces.* Paris : Presses Universitaires de France.

GUYÉNOT, E. 1946. *Les Problèmes de la Vie.* Pp. 291. Geneva : ed. du Cheval Ailé.

GUYÉNOT, E. 1942, 1948. *L'Hérédité.* 4th ed. Pp. vi+726. Paris : C. Doin et Cie (Encyclopédie Scientifique).

HAASE-BESSEL, G. 1941. *Der Evolutionsgedanke in seiner heutigen Fassung.* Pp. 76. Jena : G. Fischer Verlag.

HAGEDOORN, A. L. 1948 (1939-46). *Animal Breeding.* 2nd ed. Pp. 367. London : Crosby Lockwood (1st ed. Pp. 304).

HALDANE, J. B. S. 1941-42. *New Paths in Genetics.* Pp. 206. London : Allen & Unwin.

HALDANE, J. B. S. 1943. *Heredity and Politics.* Pp. 185. London : Allen & Unwin.

HARLAND, S. C. 1939. *The Genetics of Cotton.* Pp. 193. London : Jonathan Cape.

HARMIA, A. 1944. *Koteläinjalostuksen oppikirja* (*Textbook of Animal Genetics*). Pp. 196. Helsinki : Porvoo.

HARTMANN, M. 1943. *Die Sexualität.* Pp . x+426. Jena : Gustav Fischer Verlag.

HARWOOD, W. S. 1941. *New Creations in Plant Life. An Authoritative Account of the Life of Luther Burbank.* 1st London ed. Pp. xiv+430. London : MacMillan & Co. (1st ed. New York, 1907).

HAYES, H. K., AND IMMER, F. R. 1942. *Methods of Plant Breeding.* Pp. xii+430. New York : McGraw Hill.

HEBERER, C. (ed.). 1943. *Die Evolution der Organismen, Ergebnisse und Probleme der Abstammungslehre.* Pp. x+774. Jena : Gustav Fischer Verlag.

HINSHELWOOD, C. N. 1946-47. *The Chemical Kinetics of the Bacterial Cell.* Pp. x+284. Oxford : Clarendon Press.

HOFSTAEDTER, R. 1944. *Social Darwinism in American Thought, 1860-1915.* Pp. viii+191. Philadelphia : University of Pennsylvania Press.

HOGBEN, L. T. 1939 (1933). *Nature and Nurture.* Pp. 143. London : Williams and Norgate.

HOGBEN, L. T. 1946. *An Introduction to Mathematical Genetics.* Pp. xii+260. New York : W. W. Norton & Co.

HUDSON, P. S., AND RICHENS, R. H. 1946. *The New Genetics in the Soviet Union.* Pp. 88. Cambridge : I.A.B.

HUTCHINSON, J. B. *et al.* 1947. *The Evolution of* Gossypium. Pp. xi+160. Oxford : Oxford University Press.

HUXLEY, J. S. (ed.) 1940. *The New Systematics.* Pp. 583. Oxford : Oxford University Press.

HUXLEY, J. S. 1942. *Evolution, the Modern Synthesis.* Pp. 645. London : Allen & Unwin.

INTERNATIONAL CONGRESS OF GENETICS. 1941. *Proceedings of the 7th International Genetical Congress. 23-30 August 1939.* Pp. 336. Cambridge : Cambridge University Press.

INTERNATIONAL CONGRESS OF GENETICS. 1949. *Proceedings of the 8th International Congress of Genetics. 7-14 July 1948.* Pp. 696. Lund : Hereditas.

JACOBSEN, O. 1947. *Heredity in Breast Cancer.* Pp. 306. Copenhagen : E. Munksgaard.

JEAN-BLAIN, M. 1945. *Génétique Générale.* (Vol. 1 of *Traité de Zootechnique Générale.*) Paris.

JEPSEN, G. L., SIMPSON, G. G., AND MAYR, E. (ed.). 1949. *Genetics, Paleontology and Evolution.* Pp. 470. Princeton : Princeton University Press, New Jersey.

JOLLOS, V. 1939. *Grundbegriffe der Vererbungslehre, insbesondere Mutation, Dauermodifikation und Modifikation.* Pp. 110. (Bd. 1. Hb. d. Vererbungswissenschaften.) Berlin.

JONES, F. W. 1943. *Habit and Heritage.* Pp. 100. London : Routledge.

JUCCI, C. 1944. *Introduzione allo Studio della Genetica, per Medici, Agrari e Naturalisti.* Pp. xxiii+419. Milano : Ulrico Hoepli.

JULL, N. A. 1940 (1932). *Poultry Breeding.* 2nd ed. Pp. xv+484. New York : J. Wiley & Sons.

KALMUS, H. 1948. *Genetics.* West Drayton : Pelican Books.

KAPPERT, H. 1948. *Die vererbungswissenschaftlichen Grundlagen der Pflanzenzüchtung.* Pp. 244. Berlin : Paul Parey.

KEITH, SIR A. 1946. *Essays on Human Evolution.* Pp. x+224. London : Watts & Co. American ed. 1947. *Evolution and Ethics.* Pp. ix+246. New York : Putnam.

KEITH, SIR A. 1948. *A New Theory of Human Evolution.* Pp. 461. London : Watts & Co.

KELLEY, R. B. 1946. *Principles and Methods of Animal Breeding.* Pp. viii+296. Sidney : J. Wiley & Sons.

KEMP, T. 1943. *Arvelighedslaere for Studerende og Laeger* (*Textbook of Genetics for Medical Students*). Pp. 285. Copenhagen : E. Munksgaard.

KIHN, B., AND LUXENBURGER, H. 1940. *Handbuch der Erbkrankheiten.* Leipzig.

KIRCHMAIR, H. 1941. *Erbfibel. Grundbegriffe der Vererbungslehre* (sewn). Leipzig.

KNAYSI, G. 1944. *Elements of Bacterial Cytology.* Pp. xii+209. New York.

KNIGHT, R. L. 1948. *Dictionary of Genetics.* Pp. xi+183. Waltham : Chronica Botanica.

KOBEL, F. 1947. *Vererbung und Leben.* Pp. 216. Zürich : Büchergilde Gutenberg.

KOMAROV, V. 1940. *The Doctrine of Species* (in Russian). Pp. 212. Moscow : Acad. Scient. Ud. S.S.R. Moskwa-Leningrad.

KOLTZOFF, N. K. 1939. *Les Molécules Héréditaires.* Paris.

KÜHN, A. 1939. *Grundriss der Vererbungslehre.* Pp. 164. Leipzig : Quelle & Meyer.

LACK, D. 1947. *Darwin's Finches.* Pp. x+208. Cambridge : Cambridge University Press.

LAMY, M. 1944. *Les Applications de la Génétique à la Médecine.* 2nd ed. Pp. 146. Paris : G. Doin et Cie.

LANGDON-DAVIES, J. 1949. *Russia Puts the Clock Back.* Pp. 160. London : V. Gollancz.

LAWRENCE, W. J. C. 1937-45. *Practical Plant Breeding.* Pp. 150. London : Allen & Unwin.

LEA, D. E. 1946. *Actions of Radiations on Living Cells.* Pp. xii+402. Cambridge : Cambridge University Press.

LI, CHING LUN. 1948. *An Introduction to Population Genetics.* Pp. 321. Peiping : National University of Peking.

LINDENOV, H. 1945. *The Etiology of Deaf-Mutism with Special Reference to Heredity.* Pp. 268. Copenhagen : E. Munksgaard.

LINDQUIST, B. 1948. *Genetics in Swedish Forest Practice.* Pp. 173. Stockholm : Sv. Skogsvardsforskeningens Forlag.

LOEB, L. 1945. *The Biological Basis of Individuality.* Pp. xiii+711. Springfield, Ill. : Ch. C. Thomas.

LULL, R. S. 1947. *Organic Evolution.* 2nd ed. Pp. 764. New York : MacMillan & Co.

LUNDSGAARD, R. 1944. *Leber's Disease.* Pp. 306. Copenhagen : Nordisk Forlag.

LUSH, J. A. 1938, 1943. *Animal Breeding Plans.* Pp. viii+350. Ames, Iowa : Collegiate Press.

LYSENKO, T. D. 1943. *On Inheritance and Its Changeability* (in Russian). Moscow : Socialist Agriculture, 1/2, 3/4.

1946. *Heredity and Its Variability* (translation of above by Th. Dobzhansky). Pp. 65. New York : King's Crown Press.

LYSENKO, T. D. 1948. *Soviet Biology.* Pp. 51. London : Birch Books, Ltd.

(LYSENKO, T. D.) 1949. *The Situation in Biological Sciences.* Proceedings of the Lenin Academy of Agricultural Sciences, U.S.S.R., Session July-August 1948. Verbatim Report. Pp. 631. Moscow : Foreign Languages Publishing House. London : Collett.

MAGUINESS, O. D. 1940. *Environment and Heredity.* Pp. 216. London : Nelson.

MALECOT, G. 1948. *Les Mathématiques de l'Hérédité.* Pp. vi+65. Paris : Masson et Cie.

MARTIN, A. 1947. *Introduction to Genetics of* Habrobracon juglandis *Ashmead.* Pp. 217. New York : Hobson Book Press.

MATHER, K. 1943-46. *Statistical Analysis in Biology.* Pp. 267. London : Methuen.

MATHER, K. 1949. *Biometrical Genetics.* Pp. ix+162. London : Methuen.

MATTHEW, W. D. 1939. *Climate and Evolution.* 2nd ed. Pp. 223. New York : N.Y. Acad. Sciences.

MATTHEY, R. 1949. *Les Chromosomes des Vertebres.* Lausanne : F. Rouge.

MAYR, E. 1942, 1944, 1947. *Systematics and the Origin of Species.* Pp. xiv+334. New York : Columbia University Press.

METHORST, H. K., AND SIRKS, M. J. 1948. *Het Bevolkingsvraagstuk* (*Population Problems*). Pp. 230. Amsterdam : Scheltena en Holkema.

MEYER, F. 1947. *L'Accélération évolutive. Essai sur le Rythme évolutif et son interpretation quantique.* Pp. 68. Paris : Libraire des Sciences et des Arts.

MITTMANN, O. 1940. *Erbbiologische Fragen in mathematischer Behandlung.* Berlin.

MONTALENTI, G. 1939. *Elementi di Genetica.* Bologna.

MŒLLENBACH, C. J. 1947. *Medfødte Defekter i Øjets indre Hinder, Klinki og Arvelighedsforhold* (*Congenital Defects of the Internal Membranes of the Eye. Clinical and Genetical Aspects*). Pp. 164. Copenhagen : E. Munksgaard.

MORANT, G. M. 1939. *The Races of Central Europe.* (Preface by J. B. S. Haldane.) Pp. 163. London : Allen & Unwin.

MOERCH, E. T. 1941. *Chondrodystrophic Dwarfs in Denmark.* Pp. 200. Copenhagen : E. Munksgaard.

MOTTRAM, V. E. 1944. *The Physical Basis of Personality.* West Drayton : Pelican Books.

MOULTON, F. R. (ed.). 1940. *The Genetics of Pathogenic Organisms.* Pp. 90. Lancaster : Science Press.

MOULTON, F. R. (ed.). 1940. *The Cell and Protoplasm.* Pp. 205. Washington : American Association Advancement of Science.

MULLER, H. J. 1939. *Bibliography on the Genetics of* Drosophila. Pp. 132. Edinburgh : Oliver & Boyd (Imperial Bureau of Animal Genetics).

MULLER, H. J., LITTLE, C. C., AND SNYDER, L. H. 1947. *Genetics, Medicine and Man.* Pp. viii+158. New York : Cornell University Press.

MUELLER, W. 1939. *Die angeborenen Fehlbildungen der menschlichen Hand.* Leipzig : G. Thieme Verlag.

NATIONAL CATTLE BREEDER ASSOCIATION. 1947. *British Pedigree Cattle.* Pp. iv+110. London : Published by the Association.

NEILSON JONES, W. 1946. *Plant Chimæras and Graft Hybrids* (translated by F. Freier). Pp. 157. Buenos Aires.

NEWMAN, H. H. 1940. *Multiple Human Births.* New York.

1942. *Twins and Supertwins.* English edition. Pp. 164. London : Hutchinson.

NICHOLS, J. E. 1944. *Livestock Improvement in Relation to Heredity and Environment.* Pp. vii+208. Edinburgh : Oliver & Boyd.

OLIVER, C. P. 1945. *Collections of Records in the Study of Human Heredity.* Pp. 34. Minneapolis : University of Minneapolis.

ORTIZ-PICON, J. M. 1947. *Citologia General. Los Fundamentos citologicos de la Biologia.* Pp. ix+368. Rio de Janeiro : Editional Labor, S.A.

OSBORN, F. 1940. *Preface to Eugenics.* Pp. xi+312. London : Harper & Brothers.

PATTERSON, J. T. 1940. *Studies on the Genetics of* Drosophila. Pp. xi+256.

1942. *Gene Variation and Evolution.* Pp. xi+256.

1943. *The* Drosophilidæ *of the South West.* Pp. vii+200. Austin, Texas : University of Texas Publications.

PENNSYLVANIA UNIVERSITY. 1941. *Bicentennial Conferences on Cytology, Genetics and Evolution.* Pp. v+168. Philadelphia : University of Pennsylvania Press.

PRENANT, M. 1946. *Darwin.* Paris : ed. Hier et Aujourd'hui.

PRENANT, M. 1948. *Biologie et Marxisme.* Pp. 335. Paris : ed. Hier et Aujourd'hui.

PINCHER, C. 1946. *The Breeding of Farm Animals.* Harmondsworth : Pelican Books.

RENSCH, B. 1947. *Neuere Probleme der Abstammungslehre. Die transspezifische Evolution.* Pp. vi+407. Stuttgart : Ferd. Enke Verlag.

RICE, V. A. 1942. *Breeding and Improvement of Farm Animals.* Pp. xx+750. New York : McGraw Hill.

RICHHARIA, R. H. 1945. *Plant Breeding and Genetics in India.* Pp. 403. Patna, India.

RILEY, H. P. 1948. *Introduction to Genetics and Cytogenetics.* Pp. ix+596. New York : John Wiley. London : Chapman & Hall.

ROBERTIS, E. P. D. DE *et al.* 1948. *General Cytology* (translated by W. Andrew). Pp. 375. Philadelphia : W. B. Saunders.

ROUSSEAU, J. 1945. *L'Hérédité et l'Homme.* Pp. 250. Quebec.

RUEDIN, E. n.d. (*circa* 1947). *Studien über Vererbung und Entstehung geistiger Störungen.* Berlin.

SALAZAR, Z. 1947. *Indicion sintetica al estudio de la Genetica zootecnica, mejora del ganado y sus Problemas biologico-mathematicos.* Madrid.

SANSOME, F. W., AND PHILP, J. 1932-39. *Recent Advances in Plant Genetics.* Pp. x+414. London : Churchill.

SARKAR, S. S. 1941. *Aims and Objects of Eugenic Researches in Bengal.* Calcutta : Indian Eugenic Society.

SAULI, J. 1947. *Kasvinjalostuksen perusteista ja menetelmistä* (*Principles and Methods of Plant Breeding*). Pp. 139. Helsinki : Porvoo.

SCHEINFELD, A. 1939-45. *You and Heredity.* Pp. xix+434. London : Chatto & Windus.

SCHIFF, F., AND BOYD, W. C. 1942. *Blood Grouping Technique.* Pp. xiv+248. New York : Interscience Publ.

SCHINZ, H. R. *et al.* 1948. *Die Vererbung des Krebses beim Menschen.* Pp. 232, and 125 additional tables. Zürich : Arch. Jul. Klaus Stiftung.

SCHMALHAUSEN. 1949. *Factors of Evolution.* Philadelphia : Blakiston Co.

SCHNARF, K. 1941. *Vergleichende Cytologie des Geschlechtsapparates der Kormophyten.* Pp. 249. Berlin : Bornträger.

SCHRADER, F. 1944. *Mitosis. The Movements of Chromosomes in Cell Division.* Pp. x+110. New York : Columbia University Press.

SCHROEDINGER, E. 1944. *What is Life? The Physical Aspect of the Living Cell.* Pp. viii+91. Cambridge : Cambridge University Press.

1946. *Was ist Leben? Die lebendige Zelle mit den Augen des Physikers betrachtet.* Pp. 143. Sammlung Dalp.

SEEDORFF, K. S. 1949. *Osteogenesis Imperfecta.* Pp. 229. Aarhus : Universitetsforlaget.

SENTEIN, P. 1941. *L'Action des Toxiques sur la Cellule en Division.* Pp. 252. Montpellier.

SHARP, L. W. 1943. *Fundamentals of Cytology.* Pp. 280. New York : McGraw Hill.

SHULL, A. F. 1926-48. *Heredity.* 4th ed. Pp. ix+311. New York and London : McGraw Hill.

SILOW, R. A. 1947. *Genetics of Species Development in the Old World Cottons.* London : Empire Cotton Station.

SIMPSON, G. G. 1944. *Tempo and Mode in Evolution.* Pp. xviii+237. New York : Columbia University Press.

SINNOTT, E. W., AND DUNN, L. C. 1925-39. *Principles of Genetics.* Pp. xiv+408. New York and London : McGraw Hill.

SIRKS, M. J., AND KASTEIN, G. W. 1942. *Geneeskunde en Erfelijkeid* (*Medicine and Heredity*). 2nd ed. Pp. 352. Lochem : De Tijdstroom.

SIRKS, M. J. 1946. *Het Geslacht, Nitingen en Orzaken* (*Sex, Appearance and Causes*). Pp. 176. Gorinchem : Noordenyn.

SIRKS, M. J. 1948. *Handboek der algemeene Erfelijkedsleer* (*Textbook of General Genetics*). 4th ed. Pp. 640. Den Haag : M. Nijhoff.

SJOERGREN, T. 1948. *Genetic, Statistical and Psychiatric Investigations on a West Swedish Population.* Copenhagen : E. Munksgaard.

SNEDECOR, G. W. 1937-46. *Statistical Methods Applied to Experiments in Agriculture and Biology.* Pp. xvi+485. Ames : Collegiate Press, Inc.

SNYDER, L. H. 1935-46. *The Principles of Heredity.* Pp. 452. New York : D. C. Heath & Co.

SNYDER, L. H. 1941. *Medical Genetics.* Pp. 140. Durham : Duke University Press.

STERN, C. 1949. *Principles of Human Genetics.* New York : W. H. Freeman & Co.

STOCKARD, C. R. *et al.* 1941. *Genetics and Endocrine Basis for Differences in Form and Behavior.* Pp. xx+775. Philadelphia : Wistar Institute.

STORCH, O. 1948. *Die Sonderstellung des Menschen in Lebensabspiel und Vererbung.* Pp. vi+62. Vienna : Springer Verlag.

STURTEVANT, A. H., AND BEADLE, G. W. 1939. *Introduction to Genetics.* Pp. 391. Philadelphia and London : W. B. Saunders.

SZONDI, L. 1948. *Schicksalsanalyse.* Pp. 422. Basle : Benno Schwab & Co.

TIMOFEEFF-RESSOVSKY, N. W., AND ZIMMER, K. G. 1947. *Das Treffprinzip in der Biologie.* Pp. xii+317. Leipzig : S. Hirzel Verlag (Biophysik, Bd. 1).

VIDEBAEK, A. 1947. *Heredity in Human Leukemia and Its Relation to Cancer.* Pp. 279. Copenhagen : E. Munksgaard.

WADDINGTON, C. H. 1939. *Introduction to Modern Genetics.* Pp. 441. London : Allen & Unwin.

WADDINGTON, C. H. 1940. *Organisers and Genes.* Pp. x+160. New York and London : Cambridge University Press. New York : MacMillan & Co.

WALTER, E. H. 1913-47. *Genetics.* 4th revised ed. Pp. xvii+412. New York : MacMillan & Co.

WANSCHER, J. H. 1942. *Arvelighedsordbog* (*Glossary of Genetic Terms*). Pp. 131. Copenhagen : G. E. C. Gads Forlag.

WELLENSIEK, S. J. 1943. *Grondslagen der allgemeene Plantenveredeling* (*Principles of General Plant Breeding*). Pp. xvi+492. Harlem : H. D. Tjeenk Willink & Zoon.

WESTERGAARD, M. 1940. *Studies on Cytology and Sex Determination in Polyploid Forms of Melandrium Album.* Pp. 131. Copenhagen : E. Munksgaard.

WESTERGAARD, M. 1948. *De nye Arvelighetsteorien in Sovjetunionen.* Pp. 80. Copen hagen : Gyldendal.

WESTERLUND, E. 1947. *Clinical and Genetic Studies on the Primary Gloucoma Diseases.* Pp. 207. Copenhagen : E. Munksgaard.

WHITE, E. G. 1940. *Principles of Genetics.* Pp. 352. St Louis : Mosby Co.

WHITE, M. J. D. 1937, 1942. *The Chromosomes.* Pp. 124. London : Methuen.

WHITE, M. J. D. 1945. *Animal Cytology and Evolution.* Pp. 383. London : Cambridge University Press.

WHITNEY, D. L. 1943. *Family Treasures. A Study of the Inheritances of Normal Characteristics in Man.* Pp. 299. Lancaster : Cattell Press.

WIENER, A. S. 1943. *Blood Groups and Transfusion.* 3rd ed. Pp. xix+438. Springfield, Ill. : C. C. Thomas.

WILLIAMSON, A. A. 1946. *Evolution.* (*Evolution and the Quantitative Principles of Progress and what they mean to-day.*) Pp. xii+270. New York : Hobson Book Co.

WILLIS, J. C. 1941. *The Course of Evolution.* (*By Differentiation or Divergent Mutation Other than by Selection.*) Pp. 215. New York and London : Cambridge University Press.

WINGE, Ø. 1945. *Arvelighedslaere* (*Textbook of Genetics*). 3rd ed. Pp. 489. Copenhagen : Nordisk Forlag.

WINGE, Ø. 1945. *Arvelighed hos Hunde* (*Genetics in Dogs*). Pp. 117. Copenhagen : Nordisk Forlag.

WINTERS, L. M. 1925, 1944. *Animal Breeding.* Pp. 399. London : Chapman & Hall. New York : John Wiley.

WINTERS, L. M. 1942. *An Introduction to Breeding Farm Animals.* Pp. 260. London : Chapman & Hall.

WOODWORTH, R. S. 1941. *Heredity and Environment.* Pp. 95. New York : Social Science Research Council.

WRIGHT, SEWALL. 1941. *Statistical Genetics in Relation to Evolution.* Pp. v+63. Paris : Herrmann & Cie.

ZIRKLE, C. 1946. *Early History of the Idea of the Inheritance of Acquired Characters and of Pangenesis.* Pp. 61. Philadelphia : American Philosophical Society.

A MUTATION FROM BLACK TO BROWN IN *MUS MUSCULUS*

MARGARET E. WALLACE

Department of Genetics, University of Cambridge

Received 11.xii.49

A BUCK, 12/76.188 i, which was bred from an intercross for brown and misty in coupling (*mb*/MB × *mb*/MB), and was itself phenotypically a non-misty black, was crossed, for purposes other than the present subject, to a misty-brown doe (mating 12/82) and a non-misty brown doe (mating E/296). By the former doe he has thrown twelve non-misty blacks to date, and by the latter one brown and twenty-four blacks.

χ^2 on 1 : 1 of the B : *b* segregation of all the buck's progeny is 33·1081 for one degree of freedom.

The simplest explanation for this performance is that the buck is homozygous for non-misty and black, and that the one brown offspring recorded is the result of a mutation of black to brown affecting one of his sperm.

Unfortunately the brown was produced amongst the first eleven of his offspring. As there was a two-thirds chance of the buck being heterozygous, the brown was not unexpected and was killed when fully classified, that is at the age of eighteen days when B/*b* was also classified. There is thus no possibility of testing the mutant gene.

REVIEWS

THE ELEMENTS OF GENETICS. By C. D. Darlington and K. Mather. London : George Allen & Unwin Ltd. 25s.

If it may be said that the science of genetics was born in the early nineteen hundreds, then in half a century there have been a great number of books on elementary genetics. Most of these have been written fairly recently and can accordingly claim to be modern. An author's main task, therefore, is not so much the inclusion of all recent knowledge contributing to the foundations of genetics, but rather its orderly arrangement in a logical sequence, and its presentation in a clear authoritative style. Added to this is the secondary task of deciding what to exclude, so that his structure is not so broad as to include material not strictly genetical, and not so high as to contain matter too complex and specialised to be called " elementary."

The Elements of Genetics by C. D. Darlington and K. Mather is an excellent example of its kind. It is a common experience of the specialist that the exposition of the foundations of his subject is both tedious and, to some extent, difficult: definition of new terms the first time they appear, avoidance of assuming knowledge natural to him but strange to his readers, explanation of the subject in the simplest rather than, perhaps, the most interesting manner—all require an unravelling and sorting-out of the knowledge clear to him as a coherent whole. *The Elements* has been set forth with great conscientiousness. An historical presentation is perhaps the more interesting to those who have observed or taken part in the history, but it often involves the explanation and finally the rejection of what are now regarded as false conceptions ; this procedure can be confusing to the mind of the student, whose first impressions are probably the most lasting. Such a presentation is here avoided, in favour of a logical description of known facts and accepted theories, except in so far as the historical presentation provides a more striking and as accurate an account. On the lesser point, that of explaining new terms as they appear, there are very few omissions, and these are rendered innocuous by the provision of a clear and comprehensive glossary.

The secondary task, that of deciding the material to be included, is perhaps the more exacting. The book is divided into three parts. The first, headed " Individuals," describes in order : mitosis and meiosis ; Mendel's experiments and linkage ; continuous variation, including polygenic systems, linkage of polygenes and their biometrical analysis ; chromosome and gene changes, and the consequences of these changes. The second, headed " Cells," touches on chemical and physiological processes of genes and molecules, with a description of the expression and interaction of genes ; it includes a chapter on the cytoplasm, with an account of plastogenes, plasmagenes, cytoplasmic equilibrium, nucleus and environment, etc. ; it describes the action and interaction of nucleus and cytoplasm in development and differentiation, and includes an account of nuclear and cytoplasmic systems with special reference to viruses and the

genetic conditions of cancer development. The third part, headed " Populations," covers a wide field ; this comprises genotypic and segregational sterility, the results of inbreeding and hybridity, and breeding systems ; selection and variability, with reference to Darwinism, and the opposing requirements of fitness and flexibility ; the properties of outbreeding, changes in genetic systems, and evolution of new breeding groups ; the growth of genes, including reference, *inter alia*, to position-effects and " super-genes." Finally, there is a chapter on man and mankind ; and a concluding chapter, co-ordinating the three-level analysis made in the body of the book, and describing some trends for the future of genetics.

It can be seen from this short summary that the structure of the book has a very broad base, and it can be suspected, therefore, that its height is relatively small. In fact, a more descriptive, though longer, title might have been " The elements of genetics and its bearing on related subjects." As such the work is not sufficiently limited to be a textbook for the junior student, nor detailed enough for the specialist who desires a deeper knowledge of his own and related subjects. It is more in the nature of an introduction to genetics, providing a stimulant to the interested amateur and a background of knowledge for the serious student. In the preface the book is described as " the first attempt to represent the whole scope of genetics, the whole of what has always been needed," and as " a survey of the whole territory of genetics." The latter is described as a continent across which " botanists and zoologists may venture to find common ground with bacteriologists and virologists " and in which the investigator of cancer, the practical stock-breeder, the physical chemist, the physiologist and the embryologist play a part. The authors hope " that many, whether wise veterans or innocent enthusiasts, who read this book, will share some of the delight we have had in writing it." The delight it has given the authors is obvious and refreshing. Apart from those to whom the title is misleading, the book should provide much pleasure to all its readers.

On smaller details than scope, there are, however, some criticisms that should be made.

Part III, Populations, represents almost half of the book. This means that the first part, which is basic and therefore the most important from the point of view, at least, of " innocent enthusiasts," is relatively short, and the material in it appears somewhat cursorily treated. There is, for example, only a brief account of linkage, with little or no information on the kinds of crosses which may show it, and no mention of the statistical methods by which it may be detected. A formulation of statistical procedure is not expected, but, if the biometrical analysis of continuous variation takes up a complete chapter, at least an indication of the necessity for statistical knowledge is expected in the chapter in which simple single-factor and linkage ratios are described. Its absence is the more surprising on consideration of the statistical work of one of the authors.

It has been stated earlier that a book on elementary genetics should be presented in a clear, authoritative style. This is not to say that a writer may make sweeping or unverified statements. A few examples will suffice to show that the book under review is not flawless in this respect : p. 210, " There are many types of genetic change, in which viruses, like bacteria, react so characteristically to a particular type of change of host or diet that control seems to be indicated." This statement is not followed by one

giving another widely accepted interpretation, namely that genetic changes in viruses and bacteria, as in higher organisms, are probably undirected environmentally, but only those are observed that can be discerned in a particular environment. Again, p. 253, " Courtship behaviour in fishes, birds and mammals elaborately testifies to the (same) exercise of discrimination " (in choosing a mate). No mention is made of the school of thought which describes the primary function of courtship as that of ensuring synchronisation of male and female in sexual behaviour and in the rearing of progeny. There is much evidence for this among birds, where " courtship display " often takes place *after* the selection of a mate ; in fact many ornithologists would describe sexual selection as a secondary function of courtship, and one which is not universally operative. Finally, p. 251, " Tristyly in *Lythrum* (or in *Oxalis* or *Narcissus*) could scarcely be worked on the basis of a series of multiple allelomorphs, and, in fact, it depends on two unlinked genes." The statement is true of *Lythrum*, but it is certainly not true of *Oxalis*, where in *O. valdiviensis* the short/non-short locus is linked to that of the mid/long with a recombination value of about 5·7 per cent. Also, so far as the present reviewer is aware, no genetical results have been published on work done on tristyly in *Narcissus*.

In their preface the authors confess that they " do not hope to satisfy the critic who prefers the small, the simple, and the secluded, department." This may well be so ; but an equally important subject for hope is that they will satisfy the critic who prefers a scientific work to remain scientific, and not to include the authors' personal political or philosophical views. The statement on p. 354 beginning " our genetic principles, therefore, favour neither the extreme advocate of racial purity nor equally the extreme anthropologists, philosophers and historians (whether liberal, Marxist or Catholic) who dogmatically assert what they desire to believe . . ." is, whether or not it is true in its implications, an unfortunate lapse in a book otherwise excellent as a technical work.

The preface also provides a warning to the reader that he must " don his doubting glasses at the point he feels proper." A decision upon the point which is proper is no doubt easy for the " wise veteran," but it may be difficult for the " innocent enthusiast " for whom also the book is written. The book will doubtless, however, provide stimulation for the latter, and, for the veteran, the pleasure peculiar to deciding on what subjects the authors, as they phrase it, have " travelled too fast and too far."

Space in this review has necessarily been devoted more to unfavourable than to favourable criticism. It must be left to the reader to appreciate all that is admirable, and he will no doubt realise that the reviewer has found it difficult, not to reveal matter for commendation, but to find lapses unworthy of the general excellence of the book. It contains a wealth of up-to-date information, many useful drawings and diagrams—several of which present their facts in a new and delightfully clear and concise manner—and even some valuable opinions of other geneticists, opinions which one may reasonably suspect would never be published in any other way. Perhaps the greatest value of the book lies in the fact that it is the vehicle of much original thought on the part of its two authors, both of whom have contributed much in the past to the science of genetics.

MARGARET E. WALLACE.

LES CHROMOSOMES DES VERTÉBRÉS. By R. Matthey, professeur ordinaire à l'Université de Lausanne.

Robert Matthey for more than twenty years has been engaged on chromosome studies of vertebrates. He is thus well qualified to survey the field of vertebrate cytology. In his book Professor Matthey deals first with the all-important preservation of the natural structures of the cell. Then follow some witty pages about the technique of observation. There is a lot of truth to be found here. Preconceived ideas and hasty conclusions have often led to most remarkable " discoveries."

Matthey's work can conveniently be divided in two parts ; the sex chromosomes, and chromosome numbers and evolution.

The study of sex chromosomes consists in tracking down the unequal pair, their morphology and behaviour, such as mode of pairing, heteropycnosis, etc. Now we probably know the main types of sex mechanisms in vertebrates. In critically studied Anamniotes no sex chromosomes can be detected morphologically. The same is true in reptiles, but in birds and mammals distinguishable sex chromosomes are present and point to an over-all XY-XX mechanism. In birds the females carry the XY, while in mammals the males are the heterozygous sex. The pairing of the sex chromosomes in animals, on the whole, is very incompletely understood. Two facts must always be considered : (1) The phenomenon of heteropycnosis (precocious condensation) found in these chromosomes during meiotic prophase, and (2) the existence of a pairing segment, shown by the cytological and genetical evidence of crossing over. Any theory explaining the pairing processes has to include these facts.

The second part of Matthey's work concerns the possible deductions that can be made from the chromosome complements, as to the affinities between species or higher systematic units. This is a much more intricate question, involving loss and addition of whole chromosomes as well as changes in the individual chromosome, like fragmentation, fusion, inversion, translocation, duplication and deficiency. In 1916 Robertson suggested that the variations in chromosome numbers found in grasshoppers were caused by fragmentation and fusion. This means that a chromosome can break up into two or more pieces, thus increasing the number of units. Alternatively, fusion of chromosomes may reduce the number. We know to-day that the centromere is an essential part of a chromosome, and that acentric fragments must necessarily be lost. Apart from chromosomes with multiple centromeres (where the " centromere genes " are spaced along the chromosome) as in some Nematodes and Hemiptera-Heteroptera, the breaking up of a chromosome will not alter the number, since there will be only one fragment carried on, *i.e.* the centric piece. (In the above-mentioned examples the different fragments maintain their existence in the cell. They constitute the somatic chromosomes in *Ascaris*, and in Hemiptera-Heteroptera they can lead to multiple systems of X and Y's). In plant material, however, it has been demonstrated by Darlington (1939), Upcott (1937) and others, that a centromere can divide in the wrong plane, and break up a chromosome in its two arms. These new parts, with each a " centromere-half," can maintain their existence in the cell. It is true that the chromatids of such fragments can split apart except at the centromere region, thus forming an equal-armed V-shaped chromosome. But we know that this need not necessarily happen (*Campanula*). We can get

chromosomes with only one arm, strictly speaking, although they are not very common. The best evidence for centric splitting and union of chromosomes comes from comparative study of *Drosophila* species. Here we find several examples of rod-shaped chromosomes fused into V-shaped, and genetical analysis shows that the main structure of the arms of the rods corresponds with that of the V's. On the other hand we know that the development of V-shaped chromosomes from rods need not be caused by fusion—pericentric inversions and translocations can give the same result. From the comparative study of *Drosophila* species, we learn that of the gross morphological changes, the inversions slip most easily through the selective processes. (The hypothesis that a new centromere must always come from the pre-existing ones is not quite certain. Centromere chromomeres are known to have developed outside the heterochromatic centromere region, as shown by Kattermann, Müntzing, Prakken, Rhoades and Vilkomerson.)

Professor Matthey has applied the modernised Robertsonian theory on Anamniotes and reptiles, and in some cases on mammals. But the reader is left in doubt on the main issue. Is there a general tendency towards numerical reduction during evolution, or does the tendency differ in the various systematic groups? Is there an oscillation caused by polyploidy although never observed in higher vertebrates? Or is there no law for numerical variations except those of chance rearrangements?

In order to compare the different vertebrate species, Matthey has introduced the term " nombre fondamentale " (N.F.), meaning the total number of chromosome arms in the complement. If this N.F. shall mean anything at all, we must accept the hypothesis that chromosomes with only one arm exist. There are certainly many who deny that. From my own observations of the mouse I believe one can talk about strictly terminal centromeres. The difficulty of deciding this merely by looking at ordinary mitotic plates in animals with small chromosomes can be shown by an example from Matthey's own experience. Matthey (1945) : " En ce qui concern le Rat, l'inexistance d'un bras court par les chromosomes sexuels est *facile* à démontrer à condition que le matériel ait été convenablement fixé (Matthey, 1938, contra Koller and Darlington, 1934)." There can be no doubt that Matthey is wrong about this, which he also admits in 1948, after having seen a preparation made by his pupil Guenin. It is unfortunate that he did not take mouse chromosomes as an example instead. One must be very critical when determining the position of the centromere, and there will be considerable difficulties in deciding whether an arm is so short that it can be ignored, or whether it is sufficiently defined to be scored in the estimation of the N.F. In the rat the short arm of the X is not at all minute. It represents about one-fifth of the length of the long arm. I cannot help feeling that Matthey in his book has been somewhat uncritical when using old sources for determining the N.F. One should give the Robertsonian theory a fair chance !

However, Professor Matthey expresses criticism on other items. He throws some doubt upon the work of Koller (1936) and of Pontecorvo (1943). He questions Koller's count of 28 chromosomes in the grey squirrel, Cross (1931) having counted 48 in the subspecies *Sciurus carolinensis carolinensis*. I think that Cross's illustrations indicate preparations inferior to what one should use for chromosome work. I have studied the same material as Koller, and I have confirmed his findings.

With regard to Pontecorvo's remarkable results on the striped hamster, Matthey tells us that the material " a été fixé au liquide de Bouin seize ans auparavant par un missionaire voyageant en Chine." Bouin's fixative always gives a high degree of clumping of the chromosomes. This should account, according to Matthey, for Pontecorvo's findings of only $2n = 14$, the lowest number found in Eutheria ! In fact, however, the material was fixed in Zenker as well—and the fixation was made by Professor E. Hindle during the Kala Azar expedition of the Royal Society—which certainly fulfilled a mission, although one would hardly refer to the participants as missionaries. Moreover, I have lately had the opportunity to examine Dr Pontecorvo's slides, and he is certainly right about the number : *Cricetus griseus* has 14 chromosomes.

Matthey devotes several pages to the surprising discovery by Bovey and himself of a " sex trivalent " in males of *Sorex araneus* ($2n = 20+XXY$). This number was found in " at least 3 cells," but the authors cannot agree on the N.F. (Bovey 44, Matthey 46). In the British subspecies *Sorex araneus castaneus*, however, I have determined the number to be 24. There are 4 chromosomes of different sizes which may be associated in a chain, or in two bivalents. This is obviously the sex-determining mechanism.

Matthey has not tried to reduce the list of references. With regard to vertebrate chromosomes there is a tremendous lot of work done, but our true knowledge seems to be inversely correlated with the number of publications. It is high time that somebody started weeding out this overgrowth. The sense of piety is always in one's way—a work may be quite good considering the time when it was undertaken, but such an argument is no good to the student trying to orient himself in a new field of science.

On the other hand there is no excuse for leaving out the best references. In dealing with human chromosomes, Matthey still raises the question of the chromosome number in the male. Is it 47 or 48? Is the sex system XO or XY? Here an important work is left out, namely L. F. La Cour's " Mitosis and Cell Differentiation in the Blood " (*P.R.S. Edin.* B. **62** : Pt. I, 1944). The pre-myelocyte metaphase illustrated there is probably the best mitotic plate observed in Man, showing 10 median pairs, 8 subterminal and 6 terminal pairs, $2n = 48$ (N.F. = 84). It is really pointless to argue this question any further.

A general exposé of the work done on vertebrate chromosomes has never been given before. The present book will thus be welcomed both because it was needed and because it is well written. The arrangement of the different items is excellent, and the pictures and diagrams are well chosen. Professor Matthey's way of dealing with the problems is clear and thorough without being dull. His work will stimulate enquiry in a new and important field.

SYLFEST MULDAL.

GENETICAL SOCIETY OF GREAT BRITAIN

SYMPOSIUM ON BIOCHEMICAL GENETICS

ABSTRACTS of Papers read at the HUNDRED AND SECOND MEETING held at UNIVERSITY COLLEGE, LONDON, 29th to 31st MARCH 1950

INTRODUCTION TO SYMPOSIUM ON BIOCHEMICAL GENETICS

J. B. S. HALDANE, F.R.S.

Biochemical geneticists have, in the main, to answer four questions.

(1) What are the secondary effects of gene substitutions? That is to say we attempt to give as complete an account as possible of the differences between the developmental and other physiologies of various genotypes. We may be particularly interested in analysing the interaction of genes, and the interactions of genotype and environment.

(2) What are the primary actions of genes? Can we, for example, regard antigens or enzymes as primary products of gene action? If so, what group of substances are primary gene products; for antigenicity is not a satisfactory criterion?

(3) What agencies cause mutation, that is to say, *either* changes in genes which are then copied, or abnormalities in the copying process? Are the effects of ionising radiations and particles wholly, as they certainly are partly, due to labile chemical substances produced by them? What agencies are responsible for "spontaneous" mutation?

(4) What are genes biochemically, and how are they reproduced? Is the process of reproduction similar to their primary action?

These questions can also be asked regarding self-reproducing units not attached to chromosomes, whether these are gene-controlled or not. And questions (3) and (4) can be asked concerning whole chromosomes. It is to be noted that while an answer to question (1) would give us a new physiology, a new embryology, and a new psychology, the answer to question (4) might go a long way to answering the older question, what is life?

DOMINANCE AND FACTOR INTERACTION WITH SPECIAL REFERENCE TO PIGMENTATION OF THE GUINEA-PIG

SEWALL WRIGHT

Institute of Animal Genetics, Edinburgh

This paper is concerned with the interpretation of factor interactions at the level of characters for which the major variable factors are known and the effects in combination are measurable in terms of cell products (presumably more closely related to primary gene action than in the case of organismic patterns), but for which exact biochemical interpretation has not been practicable. The roles to be attributed on the one hand to merely quantitative differences in the actions of alleles (or immediate products) on reaction rates and, on the other, to persistence of allelic specificity along chains of reactions, are explored. Under the former head, two extreme modes of action are compared: (1) reactions of the bottleneck type, in which either the gene controlled agent or its substrate is a sharply limiting factor, and (2) multiplicative

reactions, in which rate depends jointly on activity of the gene controlled agent and concentration of substrate. These are connected as extremes of a single more general theory. The interactions of the colour factors of the guinea-pig are used to illustrate the problems of interpretation. It is concluded that persistent specificity as well as mere rate effects must be invoked.

THE NATURE OF THE SPECIFIC PRODUCTS OF THE HUMAN BLOOD GROUP GENES

W. R. J. MORGAN
Lister Institute, London

The classical blood group characters shown by human erythrocytes, and in certain instances by secretions and tissue fluids, are due to substances which are specific gene products. Recently some of these materials have been isolated in an essentially homogeneous state and the blood group A- and B-substances and the so-called O-substance are now known to be polysaccharide-amino acid complexes, the components of which are joined by primary chemical bonds. Within the last few months the specific gene products induced by the " Lewis " blood group genes, Le^a and Le^b have been isolated, the former in an apparently homogeneous condition. These products have likewise been found to be polysaccharide-amino acid complexes which possess a chemical make-up that is very similar to that demonstrated for the A-, B- and so-called O-substances. The group substances so far investigated which occur in a water-soluble form can, therefore, be classified as mucoids.

Each immunologically specific blood group substance and the character arising from its presence, is inherited as if controlled by a single gene. This fact has led to the idea, which is frequently stated, that the molecular structure of the gene product which gives rise to a characteristic antigenic specificity is similar to that possessed by the gene itself.

There seems, however, to be no evidence from studies with the specific blood group substances that there is a close structural likeness between the gene and the specific gene product. The group substances contain no nucleic acid and are thus chemically distinct from the chromosome, which contains a large amount of this material. The chromosome, furthermore, does not appear to possess a polysaccharide component and it is this material in the blood group gene products which appears to be responsible for their characteristic immunological specificity.

The immediate problem is to determine the precise differences in the closely related gene products discussed above. The results of an immuno-chemical approach along these lines will ultimately express in chemical terms one aspect of the specific action of the blood group genes in man, will establish certain characteristic chemical differences in individuals and might reveal a specific physiological function for these immunologically important materials.

INBORN ERRORS OF METABOLISM AFFECTING THE HUMAN RED CELL

A. NEUBERGER
National Institute for Medical Research, London

Congenital porphyria is a rare condition in which relatively large amounts of porphyrins of the series I are excreted in the urine and fæces. The normal produces mainly porphyrins of the series III, but excretes in addition minute amounts of the porphyrins of type I. The metabolic disarrangement is therefore quantitative and not qualitative. Isotope studies have given additional information about congenital porphyria, which will be discussed in some detail. Other conditions to be discussed are sickle cell anæmia, acholuric jaundice and methæmoglobinæmia.

BIOCHEMICAL GENETICS IN MAN

H. HARRIS
Galton Laboratory, University College, London

Many biochemical variations, which are in the main genetically determined, are now known in man. They vary from anomalies, such as fructosuria, alkaptonuria and taste insensitivity to the thioureas, which appear to have little effect on biological fitness, to disorders, such as phenylketonuria, congenital porphyria and juvenile diabetes, which manifest themselves as severe diseases and in which the fitness is drastically reduced.

Recent work in this field has been much concerned with the detailed biochemical analysis of the metabolic disorders present in these different conditions. New light, for example, has been thrown on the nature of the inborn " error " present in such disorders as phenylketonuria, methæmoglobinæmia, cystinuria and the Fanconi syndrome. In the first two conditions the disorder appears to be a metabolic block of the classical type. In the case of the last two disorders it is probable that some failure in the process of active reabsorption of particular metabolites in the renal tubules is responsible for the characteristic features which they present.

Other work in progress is concerned with the familial distributions, the population frequencies, and the variation in manifestation of these conditions.

THE EFFECTS OF SPECIFIC NUTRITIONAL FACTORS ON GENE EXHIBITION IN MUTANTS OF *DROSOPHILA MELANOGASTER*

C. GORDON
Institute of Animal Genetics, Edinburgh

Gordon and Sang (1941) worked this out in some detail for the mutant antennaless where it is clear that there are a number of specific substances some of which encourage and others of which inhibit exhibition of the mutants. One of the inhibitors is vitamin B_2 (Riboflavine). There are also a number of lesser genes (modifying genes) which affect exhibition and it is possible that these can be identified and related to specific substances. The complexity of exhibition has long been known in older mutants (eyeless, abnormal abdomen) but not so far traced to specific substances. Investigations show that ophthalmopedia (and perhaps also eyeless) shows a time emergence curve similar in many ways to antennaless and it is reasonable therefore to expect a similar underlying determination.

SOME ASPECTS OF REVERSE MUTATIONS IN A BIOCHEMICAL MUTANT OF *NEUROSPORA*

M. WESTERGÅRD
Genetical Institute, Copenhagen, Denmark

In an adenineless strain of *Neurospora crassa* which reverses spontaneously to wild type, growth has been studied in order to find out how the reversion rate can be influenced by physical and chemical agents : the results are discussed in relation to the concept of gene mutation.

NEUROSPORA

D. G. CATCHESIDE
Botany School, Cambridge

All biochemical mutations of Mendelian genes so far examined result in impairments to single metabolic reactions. Requirements more complex than those replaceable by single added nutrients are the result of inhibitions consequent upon

the abnormal accumulation of intermediates of normal intermediate metabolism or of derivatives of them. The mutant alleles result in the production of enzyme systems either unable to catalyse the reactions controlled by the respective wild type alleles, or able to do so under restricted or special conditions. The special conditions include restricted temperature and *p*H ranges, isolation from normal cell conditions and alteration of residual genotype. There is no case of an allele able to control a reaction different from that controlled by the wild type alleles.

AMINATION-DEFICIENT MUTANTS OF *NEUROSPORA*

J. R. S. FINCHAM
Botany School, Cambridge

Two mutant strains of *Neurospora crassa*, probably due to mutation at the same locus, appear to lack the ability to synthesise α-amino groups from ammonia, and will only grow if supplied with any one of a number of L-α-amino acids, glutamic acid, aspartic acid, alanine and ornithine being the most active. None of the α-keto analogues or D-amino acids which have been tested will promote growth. The strains show an impaired utilisation of ammonia as compared with the wild type, and tend to accumulate ammonia in the medium when supplied with nitrate. The bearing of these mutants on the general problem of amino acid synthesis is discussed.

BIOCHEMICAL GENETICS OF *ASPERGILLUS NIDULANS*

G. PONTECORVO
Department of Genetics, University of Glasgow

The biosynthesis of nicotinic acid from anthranilic acid *via* tryptophan, kynurenine and 3-hydroxy-anthranilic acid, as described in *Neurospora*, has been identified also in *Aspergillus*. However, in the latter the occurrence of nicotinicless mutants responding to anthranilic acid and 3-hydroxy-anthranilic acid, but to none of the other presumed intermediates, indicates the existence of an alternative short cut.

A number of genetically different mutants, all responding to adenosine, or adenine, or hypoxanthine, and a mutant responding to uridine or uracil, are all competitively inhibited by any one of the ribosides other than the one they require for growth. This inhibition is complete at very low molar ratios (about 2 : 1) and it operates only against the riboside and not against the purine or pyrimidine. Furthermore, the bases (other than the one effective in promoting growth) have no such inhibitory effect either against the ribosides or the bases.

Citrulline is not an intermediate in the synthesis arginine in *Aspergillus*. None of the arginineless strains responding to ornithine, or to ornithine and proline, respond to citrulline.

All the arginineless strains are competitively inhibited by lysine, and all the lysineless by arginine at molar ratios of about 2 : 1 for complete inhibition. The inhibition by lysine of those strains which can utilise also ornithine or proline operates only against arginine and ornithine, but not against proline. A double requiring strain lysineless and ornithineless (obtained by crossing), grows only within a very narrow range of molar ratios of the two growth factors.

AN EFFICIENT TECHNIQUE FOR THE ISOLATION OF AUXOTROPHIC MUTANTS OF MICRO-ORGANISMS

K. D. MACDONALD and G. PONTECORVO
Department of Genetics, University of Glasgow

Similarly to what Fries found in *Ophiostoma*, the conidia of mutants of *Aspergillus nidulans* with multiple growth factor requirements survive longer in a medium without these growth factors than conidia from strains with single requirements.

This differential survival we have used in developing a technique for the isolation of auxotrophic mutants. The technique, which yields up to 40 per cent. mutants, surpasses in efficiency any of those previously available.

The conidia of a biotinless strain are irradiated and plated embedded in minimal medium. After a number of days the plates are enriched with complete medium and all the colonies developing thereafter are isolated and tested for growth factor requirements additional to that of biotin present in the strain. Results obtained after irradiation with 50,000 *r.* (X-rays) show the proportion of double or multiple auxotrophs to rise from about 3 per cent. of all the colonies when the complete medium is added immediately after plating, to about 40 per cent. when the complete medium is added after 140 hours. A classification of 97 mutants shows that, besides biotin which all require, 9 require vitamins, 10 a component of nucleic acids, 76 a component of casein hydrolysate and 2 unknown substances. Compared with 22 vitamin requiring, 13 nucleic acid requiring and 35 casein requiring mutants obtained by various workers in this laboratory by " total isolation " techniques, the new technique shows a shortage of mutants of the first two types relative to the third.

The mechanism of differential survival (which may have evolutionary significance) is being investigated. The new technique lends itself to the isolation of mutants of a required type.

ANTIGEN VARIATION IN VARIETY 1 OF *PARAMECIUM AURELIA*

G. H. BEALE
Institute of Animal Genetics, Edinburgh

Previous work by Sonneborn and collaborators has indicated a fundamental difference between the genetical systems of two groups of varieties of *Paramecium aurelia*. In one group, including variety 4, hereditary cytoplasmic variations were found, whilst in the other group, to which variety 1 belongs, the more familiar Mendelian type of hereditary mechanism was found.

In the present investigation antigenic variations in variety 1 are compared with those of variety 4. Contrary to expectation, the two varieties show similar behaviour. Thus in variety 1 each stock investigated is able to develop a whole range of antigen types, depending upon genotype, external conditions (especially temperature), and to some extent on the parental cytoplasm. Differences of detail are, however, apparent : *e.g.* in variety 1 it appears that each antigenic type within a stock has its characteristic optimum temperature.

STRAIN DIFFERENCES IN RESPONSE OF MICE TO VITAMIN B DEFICIENCY

I. Activity of the Succinoxidase Enzyme System

L. A. ELSON
Chester Beatty Research Institute, London

Mice deprived of vitamin B_1 (Aneurin) show decreased activity of the liver succinoxidase owing to depletion of the cytochrome C component of this enzyme system (Elson, 1947). This effect, which was first observed in MRC mice, has been examined in the following pure lines : Strong A, C 57, ABC, CBA, C_3H.

In MRC mice there is a considerable variation both in the survival time of the animals on the deficient diet and in the intensity of the enzyme effect measured at a standard time after commencement of feeding the Aneurin deficient diet. The pure line mice show very much less variation in these effects amongst animals of the same strain, but marked differences in response between the various lines are found. This response can be assessed by means of a " deficiency index." MRC

and Strong A mice show the highest index (greatest response) and C 57 the least. The possibilities of assessing the influence of genetic factors on nutritional behaviour by this type of approach will be considered.

STRAIN DIFFERENCES IN RESPONSE OF MICE TO VITAMIN B DEFICIENCY

II. Meiosis and Sperm Differentiation

P. C. KOLLER

Chester Beatty Research Institute, Royal Cancer Hospital, London, S.W. 3

Spermatogenesis in mice fed on vitamin B_1 deficient diet displays various cytological abnormalities, such as suppression of sperm differentiation, the formation of giant or multinucleate cysts in the seminiferous tubules, spindle and chromosome disturbances, etc. A comparative analysis of various strains of mice has shown differences within and between strains in respect of the time of onset of the cytological disturbances and of the type of abnormalities induced. The cytological differences are correlated with the differences in enzyme effects (see previous paper), which have been revealed by a uniform treatment, in the present case by exposure to vitamin B_1 deficient diet. Thus the strain-differences have a genetical basis ; the differences might also have been accentuated by selection, carried out for a particular purpose, *e.g.* high or low cancer incidence. Evidence was obtained to show that a temporary depletion of vitamin B_1 can lead to permanent genetical changes which may be transmitted. These findings, together with those obtained on the Golden Hamster (Koller, 1946), indicate one possible mechanism by which the naturally occurring variations could arise.

CYANOGENESIS IN WHITE CLOVER

J. L. FYFE and A. K. CHAKRAVARTY

Plant Breeding Institute, School of Agriculture, Cambridge

Some white clover plants produce prussic acid on autolysis. This property (cyanogenesis) has been used in identifying strains of white clover and has been suspected by Evans and Evans of causing bloat in grazing cattle. Melville *et al.* have studied the biochemistry of cyanogenesis ; its genetics have been studied by R. D. Williams, by Corkill and by Atwood. Broadly, the results of these studies establish the following conclusions : (i) the production of enough prussic acid to be detected by picrate paper requires the presence of cyanogenetic glucosides (*linamarin* and *lotanstralin*) and of a specific, hydrolytic enzyme, *linamarase* : (ii) in acyanogenetic plants, glucoside is present but below a threshold value ; (iii) a Mendelian factor at one locus is concerned in the production of sufficient glucoside ; (iv) another Mendelian factor at a second locus, independent of the first, is concerned in the production of enzyme. The occurrence, however, of aberrant ratios, of unexpected types in hybrid progenies and of difficulties in classifying certain individuals suggest that the genetical explanation is not yet complete.

Data from population studies, from hybrid progenies and from repeated testing of individuals will be presented and discussed.

AN ANALYSIS OF THE MUTAGENIC ACTION OF FORMALIN

C. AUERBACH and H. MOSER

Institute of Animal Genetics, Edinburgh

The mutagenic effect of formalin in the food of *Drosophila* depends to a remarkable degree on age and sex of the treated germ cells, and on the composition of the

culture medium. Attempts to induce mutations by pre-treating individual food constituents with formalin have led to positive results. Formalin induced mutations comprise all types: lethals, visibles, small and large rearrangements; delayed mutations are frequent.

THE PRODUCTION OF MUTATIONS IN *DROSOPHILA* USING TWO CLASSES OF COMPOUNDS, THE "AROMATIC NITROGEN MUSTARDS" AND THE EPOXIDES

J. BIRD

Chester Beatty Research Institute, London, S.W. 3

Five compounds have been tested for their mutagenic action on *Drosophila melanogaster*:—

(*a*) NN-Di-(2-chloroethyl)-*p*-toluidine.
(*b*) NN-Di-(2-chloroethyl)-*p*-anisidine.
(*c*) β-Naphthyldi-(2-chloroethyl) amine.
(*d*) β-Naphthyldi-(2-chloropropyl) amine (mixture of isomers).
(*e*) 1 : 2, 3 : 4-diepoxybutane.

All five were used in feeding experiments, being mixed with the food on which a counted number of larvæ were reared. (*e*) was also injected into adult males and (*b*) was given as an aerosol to adult males. A dosage level killing about half the adult or larvæ was used in most experiments. Treated males were tested for sex-linked recessive lethals by the Muller-5 technique.

In the feeding experiments all five compounds produced a small although significant increase in the lethal mutation rate, *i.e.* 0·8-3·8 per cent., controls 0·2 per cent.; the lethals being more frequent in sperm produced during the early days of adult life. The (*e*) injected males gave 10·3 per cent. lethals and also 0·6 per cent. visible sex-linked recessives in the same experiment, the peak of lethal production being 5-9 days after injection. Preliminary aerosol experiments indicate lethals are produced about 6 days after exposure.

Males reared on (*a*) and (*c*) were also tested for the production of fragments and visibles. Only one fragment and 2 visible sex-linked recessives were found but both compounds gave about 1 per cent. Minutes, which were probably mainly small deficiencies. Many of the lethals produced in the feeding experiments have been placed genetically and show several clusters from individual males, indicating that early germ cells rather than mature sperm were affected.

EXPERIMENTAL WORK WITH AMPHIBIAN OOCYTE NUCLEI

H. G. CALLAN

Institute of Animal Genetics, Edinburgh

The mature oocytes of various amphibians contain large nuclei which can be isolated freehand under a binocular microscope. The structure and properties of the nuclear membrane have been studied by means of the electron microscope and ancillary techniques. The colloidal and chemical properties of the nuclear sap have been studied by various techniques including U/V absorption and paper chromatography. A study of chromosome structure in the electron microscope has just begun and it has already yielded results of some interest.

CONTRIBUTORS TO VOLUMES 1, 2 AND 3

AUSTRALIA
- Burnett, F. M.
- Fenner, F.
- Montgomery, C.
- Smith-White, S.
- Walsh, R. J.

BELGIUM
- Heuts, M. J.

DENMARK
- Ditlevsen, E.
- Kemp, Tage
- Winge, Ø.

FINLAND
- Pesola, V. A.

FRANCE
- L'Héritier, Ph.

GERMANY
- Pätau, K.
- Staudt, G.

INDIA
- Bhat, N. R.
- Majumdar, D. N.

ITALY
- Cavalli, L. L.
- Magni, G.
- Buzzati-Traverso, A.

NEW ZEALAND
- Boyce, S. W.
- Copp, L. G. H.
- Frankel, O. H.
- Fraser, A. S.

NORWAY
- Berge, S.

PERU
- Harland, S. C.

SOUTH AFRICA
- Moffett, A. A.

SWEDEN
- Bonnier, G.
- Hansson, A.
- Müntzing, A.

TASMANIA
- Dobie, D. L.
- Ford, J. M.
- McAulay, A. L.

U.S.A.
- Dobzhansky, Th.
- Haskins, C. P.
- Haskins, E. F.
- Lederberg, J.
- Snell, G. D.

WEST INDIES
- Dodds, K. S.
- Simmonds, N. W.

UNITED KINGDOM
- Bateman, A. J.
- Billingham, R. E.
- Crane, M. B.
- Darlington, C. D.
- Dowdeswell, W. H.
- Finney, D. J.
- Fisher, R. A.
- Ford, E. B.
- Koller, P. C.
- La Cour, L. F.
- Lewis, D.
- Mather, K.
- Medawar, P. B.
- Nutman, P. S.
- Owen, A. R. G.
- Race, R. R.
- Revell, S. H.
- Wigan, L. G.
- Yates, F.
- and others

Copies of Vols. 1, 2 and 3 are still available in 3 parts, royal 8vo. edges uncut price £3 or $10.50 net per volume

Vol. 1. Pp. 398. 13 full page plates, 80 text figs.
Vol. 2. Pp. 412. 10 full page plates, 75 text figs.
Vol. 3. Pp. 390. 13 full page plates, 51 text figs.

CONTENTS

PAGE

BILLINGHAM, R. E., and MEDAWAR, P. B. (Birmingham). Pigment spread in mammalian skin : serial propagation and immunity reactions 141

DIPPELL, RUTH V. (Indiana). Mutation of the killer cytoplasmic factor in *Paramecium aurelia* 165

SPASSKY, B., ZIMMERING, S., and DOBZHANSKY, TH. (New York). Comparative genetics of *Drosophila prosaltans* 189

SPASSKY, B., and DOBZHANSKY, TH. (New York). Comparative genetics of *Drosophila willistoni* 201

DARLINGTON, C. D., and LA COUR, L. F. (Hertford). Hybridity selection in *Campanula* 217

BEHR, ISOLDE (Hertford). Books and special volumes in the field of genetics, 1939-1949 249

WALLACE, MARGARET E. (Cambridge). A mutation from black to brown in *Mus musculus* 259

REVIEWS :

WALLACE, MARGARET E. The elements of genetics 261

MULDAL, SYLFEST. Les chromosomes des vertébrés 264

GENETICAL SOCIETY OF GREAT BRITAIN : Symposium on biochemical genetics 267